(2)

CONTRACT NO: DAMD17-90-C-0109

TITLE: ANTIDOTES FOR RICIN INTOXICATION

SUBTITLE: Effects of Ricin on the Vascular Neuroeffector System

PRINCIPAL INVESTIGATOR: Casey Robinson
AUTHORS: Victoria J. Christiansen, Chia-Hsuh Hsu, Lin Zhang

CONTRACTING ORGANIZATION: University of Oklahoma
Health Sciences Center
P.O. Box 26901, 1110 N. Stonewall
Oklahoma City, Oklahoma 73190

REPORT DATE: October 25, 1992

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	25 October 1992	Final Report (9/26/90-9/25/92)

4. TITLE AND SUBTITLE Antidotes for Ricin Intoxication
Subtitle: Effects of Ricin on the Vascular Neuroeffector System

5. FUNDING NUMBERS
Contract No.
DAMD17-90-C-0109

6110A
3M161102BS12.AB.111
WUDA335510

6. AUTHOR(S)
Casey P. Robinson; Victoria J. Christiansen; Chia-Hsuh Hsu; and Lin Zhang

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Oklahoma
Health Sciences Center
P.O. Box 26901, 1110 N. Stonewall
Oklahoma City, Oklahoma 73190

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research & Development Command
Fort Detrick
Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The i.v. 48 hour (and 7 day) LD_{50} and the minimum lethal dose (MLD) of ricin in male New Zealand White rabbits has been determined by the Up and Down procedure. A MLD and a toxic sub-lethal dose (TSD) lowered blood pressure after a 12 hour or greater lag period, but only the MLD did so significantly ($p < 0.05$). Heart rate was increased when blood pressure was reduced, which seems to be a reflex effect, but the ECG was not altered. Abnormal laboratory values correlated well with histological findings. Serum CPK, SGPT, LDH, and cholesterol concentrations were higher and serum calcium concentrations were lower in rabbits given ricin. Rabbits that died earliest (approximately 22 hours after ricin) had marked pulmonary damage, while those that died later (36-48 hours after ricin) showed much more heart and liver damage. Ricin increased total blood flow to most organs. Exceptions were the brain and lungs, where the MLD markedly

14. SUBJECT TERMS	15. NUMBER OF PAGES
Ricin; Ricin in rabbits; Ricin cardiovascular effects; Ricin vascular tissue; Ricin neuroeffector mechanism; Ricin toxicity; Ricin on blood vessels; RA I; BD	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Block 13. Abstract (con't)

reduced blood flow. Ricin administration decreased the sensitivity of the central ear artery to norepinephrine (NE) (i.e., increased the EC_{50}). Ricin increased methacholine (endothelium-dependent) relaxations of aorta rings, but did not alter those to papaverine. Ricin did not alter the activity of monoamine oxidase or catechol-*O*-methyltransferase, which metabolize NE. Ricin in some studies increased the amount of NE released by nerve stimulation, but did not alter NE reuptake by the neuronal membrane. Ricin did not alter basal calcium uptake by the aorta, but depressed stimulated calcium uptake in some studies. Ricin did not alter basal calcium efflux from the aorta, but increased stimulated calcium efflux. Thus administration of a MLD or TSD ricin markedly alters blood flow distribution, reduces blood pressure, and affects several components of the vascular neuroeffector system.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

CR Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

CR In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

___ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

___ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Casey Robinson, Ph.D.
PI - Signature

25 Oct 92
DATE

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

A. Nature of the Problem

Ricin is a toxic lectin from the castor bean (Ricinus communis) which exists in slightly different forms in seeds of different origin. Ricin D is isolated from large castor beans which originate in Thailand whereas ricin E comes from small castor beans cultivated in Japan (Hatakeyama *et al., 1989*). These differ in amino acid sequence as well as toxicity. Ricin consists of two different polypeptide chains linked together by a single disulfide bond. The B chain binds the toxin to cell surface receptors containing terminal galactose residues (Nicolson and Blaustein, 1972). This appears to be an obligatory step in the intoxication of the cell (Olsnes *et al.*, 1974 and 1976). In the entry process the disulfide bond is broken and the free A chain exhibits the toxic action on the cell by inhibiting protein synthesis (Olsnes *et al.*, 1976). The extreme toxicity of ricin is due to the fact that the liberated A chain is an enzyme and a single A chain in the cell may be sufficient to kill the cell (Eiklid *et al.*, 1980). Even though ricin is toxic to all nucleated mammalian cells tested (Olsnes and Pihl, 1982), it appears that it is more toxic to certain malignant cells (Fodstad and Pihl, 1978).

B. Background of Previous Work

1. General

Ricin and related proteins have been reviewed by Olsnes and Pihl in 1976 and 1982. In the present review, the actions of ricin are briefly reviewed, and its use in therapy is included.

2. Chemistry of Ricin

The primary structure of ricin D has been determined by Funatsu *et al.* (1978 and 1979). The sequence of the B chain was redetermined and revised in 1985 by Araki and Funatsu. There are 4 internal disulfide bridges in the B chain, but none in the A chain. There are no reactive sulfhydryl groups on the intact toxin. However, in the presence of sodium dodecyl sulfate (Cawley and Houston, 1979) and 6 M guanidine chloride (Yoshitake *et al.*, 1978) a buried sulfhydryl group can be uncovered in position 171 of the A chain. The neutral A chain in native ricin can be observed as a doublet when analyzed by polyacrylamide gel electrophoresis. These have been

designated as an A_1 component (64%) with a molecular weight of 30,000 and an A_2 component(36%) with a molecular weight of 32,000. This higher molecular weight component contains an additional second mannose-rich oligosaccharide (Foxwell *et al.*, 1985). The A chain has 265 amino acid residues with sequences consisting of both hydrophilic and hydrophobic sections, and has a helical content estimated at 0.3% (Funatsu *et al.*, 1973). The acidic B chain has 262 amino acid residues with a molecular weight of 31,557 (Araki and Funatsu, 1985) and has a helical content of 10% (Funatsu *et al.*, 1979). The B chain has four internal disulfide bridges linking cysteine residues at positions 20 to 39, 63 to 80, 151 to 164 and 190 to 207 (Araki and Funatsu, 1985). Even though weak interactions would hold the chains together, the presence of the intact disulfide bridge linking the two appears to be necessary for ricin's toxic effect. However this link must be reversible in order to release the free A chain into the cell. When a covalent interchain crossing of ricin is made with N,N'-*o*-phenylenedimaleimide, a non-toxic product results. Similar treatment of the free A chain did not alter toxicity (Oda and Funatsu, 1979). The A chain of ricin D and ricin E is identical. The amino acid sequence for the B chain of ricin E was determined by Araki and Funatsu (1987). The B chain of ricin E contains 262 amino acid residues and is composed of the N-terminal half of ricin D and the C-terminal half of Ricinus communis agglutinin, another toxic lectin found in the castor bean (Araki *et al.*, 1986).

The amount of carbohydrate in the structures is still under debate but it is clear that most of the carbohydrate in the molecule is associated with the B chain. Funatsu *et al.* (1971) and Nanno *et al.* (1975) found one oligosaccharide chain on the A chain consisting of $(GlcNAc)_2(Man)_4$. Whereas Foxwell *et al.* (1985) found one oligosaccharide chain on the A_1 component of the composition $(GlcNAc)_2(Xyl)_1(Fuc)_1(Man)_{3-4}$ and evidence for another oligosaccharide chain on the A_2 chain consisting of only GlcNAc and Man. Funatsu *et al.* (1971) found two chains on the B chain consisting of $(GlcNAc)_2(Man)_6$ and $(GlcNAc)_2(Man)_7$. These oligosaccharide chains are each attached to asparagine residues. In addition ricin binds to concanavalin A indicating that the mannose residues are exposed. Crystallization studies of ricin have been carried out. These show only one ricin molecule per asymmetric unit. A low resolution study (4 Å) showed a bilobal structure for ricin B chain with each domain being able to bind a galactose residue (Villafranca and Robertus, 1981). A further refinement of the structure of ricin to 2.5 Å has been done by the Robertus group (Rutenber *et al.*, 1991, Katzin *et al.*, 1991, Rutenber and Robertus, 1991).

Ricin can be purified by affinity chromatography on Sepharose 4B. This matrix which contains β-galactose residues, binds the ricin toxin as well as ricinus agglutinins. These can be separated by N-acetyl-galactosamine which only elutes the ricin toxin, the agglutinin requires elution by galactose (Nicolson *et al.*, 1974).

3. Mechanism of Action

It has long been known that ricin interferes with protein synthesis. In 1974 Olsnes *et al.* concluded that ricin inactivated the 60S ribosomal subunit making it unable to interact with the EF2 elongation factor. Endo *et al.* (1987) found that the target of the toxin was the rRNA as opposed to the ribosomal proteins in the 60S subunit. Their evidence showed that the 28S rRNA of the 60 S subunit had reduced mobility in electrophoretic gel after treatment with ricin. This change in migration disappeared when the samples were analyzed by gel electrophoresis in the presence of urea. These data led to the conclusion that the change in mobility was due to a conformational or chemical modification rather than a change in length of the fragment. Determination of the nucleotide sequence by enzymatic digestion revealed a missing adenine residue at position 4324 in the ricin treated ribosomes. This missing adenine rendered the fragment susceptible to digestion by nucleases. That the nucleotide sequence around position 4324 is part of a highly conserved sequence between species and the toxin is active only in eukaryotes, indicates that the toxin must recognize a specific conformation and not a nucleotide sequence. In 1987 Endo and Tsurugi quantitated the amount of adenine liberated per mole of ribosomes treated with ricin. They discovered that almost 1 mole of adenine (0.78 to 0.84 mole) was liberated per mole of ribosomes treated. They also treated ribosomes with ricin in the presence of [P^{32}]phosphate. Less than 1 mole of the phosphate was incorporated per 100 moles of the modified ribosomes. They concluded that the ricin A chain removes the adenine residue by hydrolysis and does not act as a phosphorolytic enzyme. They further concluded that ricin A chain has RNA *N*-glycosidase activity cleaving the *N*-glycosidic bond of a single adenine residue (A^{4324}) of the 28 S rRNA in a hydrolytic fashion, leaving the phosphoribose backbone intact. This removal does not require any cofactors since ricin will inactivate isolated ribosomes (Olsnes *et al.*, 1974). Site directed mutagenesis of the ricin A chain indicates that Glu177 is a key catalytic residue. A mechanism of action was proposed that involves binding of the substrate adenine in a syn configuration that resembles the transition state, with the putative oxycarbonium ion stabilized by interaction with

Glu177 (Ready *et al.*, 1991). Olsnes *et al.* (1975) showed in kinetic experiments that ricin A chains in simple buffer solution inactivated salt-washed ribosomes at a rate of about 1500 ribosomes per minute per ricin A chain. The Q_{10} was about 1.8 and the K_m about 1-2 X 10^{-7}M. The inactivation of ribosomes could be halted at any time by adding specific anti-A chain antibodies. Several plant toxins have similar structure and action to ricin. Two of these, abrin and modeccin also remove adenine from A^{4324} (Endo *et al.*, 1987). In addition to protein synthesis inhibition, ricin has other actions. Arachidonic acid metabolism is induced by ricin in cultured macrophages (Naseem and Pace, 1991). Following aerosol exposure ricin also induces the release of tumor necrosis factor α (Bavaria *et al.*, 1992), acid and alkali phosphatase, lactic dehydrogenase and 5' nucleotidase (Creasia *et al.*, 1992). The release of these compounds may be responsible for the pulmonary necrosis and some of the other toxicities noted following aerosol exposure to ricin. In addition, studies of mitochondrial function in alveolar macrophages indicate that ricin causes an inhibition of oxygen consumption in these cells and may affect the function of the electron transport chain (Swauger *et al.*, 1992).

4. Steps in Ricin Toxicity

The B chain is responsible for the binding of the toxin to the cell surface. The chain combines with galactose moieties on the cell surface. Baenziger and Fiete (1979) studied the specificity of this binding by measuring the association constant (K_a) of ricin with a series of glycopeptides of known composition. They concluded that β-1,4-linked galactose residues are primarily responsible for binding. The oligosaccharides which bind ricin can be found on a variety of glycoproteins and glycolipids. Therefore the toxin can bind to several different molecular species. Binding studies have shown that HeLa cells possess 3 X 10^7 binding sites for ricin, even though studies have shown that a single toxin molecule within the cytosol is sufficient to ensure cell death (Eiklid *et al.* 1980). Hatakeyama *et al.* (1989 and 1990) report two binding sites on the B chain, a high affinity binding site (HA) and a low affinity site (LA). The ethoxyformylation of histidine residues in ricin E abolishes the saccharide binding capability of the HA site, indicating that one of the three histidine residues must be present at the HA site.

After combining at the cell surface the B chain facilitates the entry of the A chain into the cell where it interacts with the 60S ribosomal subunit and interferes with protein synthesis (Olsnes *et al.*, 1974). The ricin is

transported into the cell complexed to the receptor in a process called receptor-mediated endocytosis. It is then stored in endosomes until it is released into the cytosol (Olsnes and Sandvig, 1983). According to work done by Moya *et al.* (1985), ricin entry into the cell is not dependent on coated pits. After hypotonic shock and incubation in K^+-free media, cells will arrest their clathrin coated pit formation (Larkin *et al.*, 1983). After this treatment the ricin still retained the original amount of cell toxicity. Sandvig and Olsnes (1982) tried several treatments in an attempt to alter the entry of ricin into the cell, in order to better understand the process. They found that lowering the pH below neutral decreases the toxicity of ricin and that at pH 6.0 the toxin was unable to inhibit protein synthesis. Also a pH of 8.0 increased the cell's sensitivity to ricin. Their studies showed an increased Ca^{2+} uptake at this higher pH. Their further studies indicate that this pH effect is not due to reduced endocytosis or reduced binding to the cell surface. Since endocytosis has been reported to be an energy dependent process (Silverstein *et al.*, 1977), Sandvig and Olsnes (1982) investigated the effect of metabolic inhibitors on the toxicity of ricin. A combination of 2-deoxyglucose, an inhibitor of glycolysis, and sodium azide, an inhibitor of oxidative phosphorylation, provided strong protection against the toxin. 2-deoxyglucose by itself will not inhibit endocytosis, but does provide some protection against the toxin. This may be due to an interference with the movement of material between different membrane compartments. Calcium in the media and its influx into the cell are not required for entry of ricin into the cell.

A deprivation of Ca^{2+} in the media provided only partial protection against ricin intoxication. Verapamil, a calcium channel antagonist, offered moderate protection against intoxication. This partial protection by Ca^{2+} deprivation and a calcium channel blocker along with the increased toxicity at higher pH where Ca^{2+} influx is increased seems to indicate that ricin is internalized by two mechanisms, one of which is Ca^{2+} dependent and another which is Ca^{2+} independent (Sandvig and Olsnes, 1982). Naseem *et al.* (1992) found that extracellular calcium was required for binding of ricin to cell surface receptors in cultured macrophages. Addition or deletion of magnesium had no effect on ricin-induced protein synthesis inhibition or binding of ricin to the cells. Sodium deprivation afforded no protection. The presence of trivalent cations of the lanthanide series provided strong protection against intoxication. These cations markedly inhibit the uptake of Ca^{2+} by the cells. However, Fe^{3+} which does not affect calcium flux, also provides good protection against ricin intoxication, so the protection afforded

by the lanthanides may come from another mechanism and not from their effects on calcium flux. These treatments do not affect the ability of the ricin A-chain to inactivate ribosomes in cell-free systems. Therefore, all of these protective effects are due to the inability of the toxin to enter the cytosol and gain access to the ribosomes (Sandvig and Olsnes, 1982). Internalization of the ricin toxin is a very slow process with only about 8% of cell-bound toxin internalized each 10 minutes at 37°C in Hep_2 cells with functional coated pits (Moya *et al.*, 1985).

After endocytosis, ricin is not easily degraded and only small amounts appear to be accumulated in lysosomes. Two hours after internalization, about 90% of internalized ricin remained intact (Sandvig *et al.* 1978). Later, part of the endocytosed ricin was released back into the medium, presumably by recycling of the receptor-toxin complex back to the cell surface (Sandvig and Olsnes, 1979). It had been suggested that ricin enters the cytosol by the rupture of endocytic vesicles (Nicolson 1974, Nicholson *et al.*, 1975). However, in the light of the work by Sandvig and Olsnes (1982) this unspecific vesicle rupture does not seem likely. They showed that low pH and absence of Ca^{2+} protected well against the toxicity of ricin and related toxins, abrin and modeccin, but not against diphtheria toxin in spite of the fact that all were endocytosed equally under these conditions. The transfer of endocytosed ricin to the Golgi complex appears to be necessary for intoxication (Sandvig *et al.*, 1986). Studies involving the labeling of ricin with a horseradish peroxidase (van Deurs *et al.*, 1986) found that ricin was routed through the vacuolar and tubulovesicular portions of the endosomal system on its way to the Golgi complex. With immunogold labeling, Hansen *et al.* (1989) demonstrated the localization of ricin in Golgi stacks and associated *trans*-Golgi network. Evidence now shows that the disulfide bond linking the A and B chain in broken somewhere in the Golgi network and ricin A chain is translocated to the cytoplasm from the *trans*-Golgi network. van Deurs *et al.* (1990) present a scheme for intracellular routing and sorting of ricin based on current knowledge and speculation. Ricin, bound to membrane glycoproteins and glycolipids, is internalized by both uncoated and coated pits and vesicles to reach endosomes. From the endosomes it may be rapidly recycled to the cell surface, transferred to lysosomes where it is slowly degraded or delivered to the *trans*-Golgi network. From the *trans*-Golgi network it can be routed back to the cell surface or translocated into the cytosol where it can inhibit protein synthesis. Goldmacher *et al.* (1992) presented evidence that the galactose binding property of ricin B chain is necessary for both membrane binding and the translocation of the A chain into the cytosol.

5. Toxicity

a. Cell Culture

Toxicity of ricin in cell culture was first shown by Lin *et al.* (1970 and 1971). The earliest toxic effect of the toxin observed is protein synthesis inhibition. Later DNA and then RNA synthesis is inhibited. There is no effect on energy metabolism or oxidative phosphorylation. Data show that protein synthesis inhibition is primarily responsible for cell death. Ricin is toxic in tissue culture in concentrations of about 1 ng/ml. A lag time is apparent after the addition of ricin to a cell culture. This lag time can be reduced by increasing the concentration of the toxin, but the lag time is always more than 20 to 30 minutes even at high concentrations (Refsnes *et al.*, 1974 and Olsnes *et al.*, 1976). The synthesis of proteins may be inhibited to different extents. For example in a myeloma cell line, the synthesis of a myeloma protein (IgA) was more rapidly inhibited than bulk protein synthesis (Ko and Kaji, 1975). Different cell lines differ in sensitivity to ricin, but this sensitivity does not correlate well between animals and cell culture. Ricin D and ricin E are equally toxic in mice, however in cell culture ricin D is much more toxic than ricin E (Koga *et al.*, 1979). After intoxication the cell undergoes early morphological changes. The cell surface becomes irregular, but the surface membrane remains functional even after all protein synthesis ceases. This is demonstrated by the cells continuing ability to exclude trypan blue (Nicolson *et al.*, 1975 and Lin *et al.*, 1970).

b. Animals

Ricin is especially toxic after parenteral administration, but even after oral administration it still exhibits toxicity. The toxicity of ricin varies among species with the guinea pig more sensitive on a weight basis than the mouse and the horse being the most sensitive animal of those tested (Ehrlich, 1957 and Balint, 1974). Fodstad *et al.* (1976) determined an approximate LD_{50} in B6D2 mice of 55 to 65 ng/mouse (mice weighing 22-26g). The distribution of ricin was determined by radiolabeling. The greatest amount of toxin was found in the spleen and liver with kidney and blood showing a lower level. In 1984, Godal *et al.* did distribution studies with radiolabeled ricin and confirmed the high levels in the spleen and liver, but also found

high levels in the bone marrow and adrenal cortex. No activity was found in the brain. They found that the sensitivity to ricin differs even between strains of the same species. These investigators felt that a minimum lethal dose (MLD) was a more meaningful parameter than an LD_{50}. The MLD in mice varied from 1.95 μg/kg for DBA mice to 2.40 μg/kg for B6D2 mice. Fodstad *et al.* (1979) found the MLD of ricin in rats, guinea pigs, rabbits and dogs to be 0.35-0.5, 0.40-0.50, 0.03-0.06, and 1.6-1.75 μg/kg, respectively. Clinical symptoms of acute intoxication included loss of appetite and body weight, slight fever, edema in the extremities and ascites. Pathological findings included enlarged and congested spleen along with the reticuloendothelial cells of the liver and spleen showing increased phagocytic activity. Hematologic parameters were altered with a decrease in hematocrit and thrombocytes and an increase in leukocytes. However bone marrow examinations in dogs revealed no clear abnormality in myelopoiesis. After sub-lethal doses of ricin the animals recovered completely.

Leek *et al.* (1989) investigated the intestinal pathology following an intramuscular dose of ricin in rats. The severity of the cellular infiltration found was similar to that found in a local immune response triggered by an orally administered toxin. Griffiths *et al.* (1987) found large scale disruption in lymphoid tissue with an apoptotic type of cell death after an intramuscular dose of ricin.

The mannose-terminal oligosaccharide of the A chain acts as a ligand for the mannose receptor in macrophages *in vitro*, leading to intoxication of the cells (Simmons *et al.*, 1986 and Skilleter and Foxwell, 1986). This allows the toxin to be removed from the blood by the reticuloendothelial system and accounts for the high levels of the toxin found in the liver by Fodstad *et al.* (1976) and the severe damage which occurs in the hepatic sinusoids as reported by Derenzini *et al.* (1987).

It has been inferred that the oral administration of ricin primarily affects the intestinal mucosa and impairs sugar absorption of the small intestine (Ishiguro *et al.*, 1983). Studies of ricin toxicity on epithelial cells indicate that the oral toxicity of ricin can be attributed to disruption of the epithelial cell membrane by the B chain and protein synthesis inhibition by the A chain leading to cell death

(Ishiguro *et al.*, 1992).

Retrograde transport of ricin in neurons has been demonstrated by Harper *et al.* (1980). Their experiments confirmed a retrograde transport of ricin from the submandibular gland of rats to neuronal bodies in the superior cervical ganglia. Morphologic changes in the neurons support the observations from biochemical studies that ricin interferes with ribosomal function and protein synthesis. A small number of the neurons are destroyed, but no phagocytosis occurs with the capsular cells remaining intact around empty spaces. In 1982 Wiley *et al.* confirmed this retrograde transport by dipping a transected nerve into a ricin solution and then determining neuronal cell changes after 12 to 52 days. They found cell damage limited to those neurons which projected into the application site of the nerve. This damage included the disappearance of virtually all Nissl substance, pycnotic nuclei and a glial reaction. Helke *et al.* (1985) reported that the retrograde transport of ricin applies only to those nerves projecting into the periphery or located in the periphery, and not to those neurons residing entirely within the CNS.

In addition to the systemic toxicities, ricin also causes pulmonary necrosis when administered by the aerosol route. Studies indicate that the pulmonary necrosis is not caused by protein synthesis inhibition by ricin, but rather by ricin-induced release of tumor necrosis factor α, interleukin-1 and various enzymes indicative of macrophage activation (Bavari *et al.*, 1992, Creasia *et al.*, 1992).

c. Man

In man, the signs and symptoms of ricin intoxication vary greatly according to the dose of toxin administered. As is always the case, the symptoms appear only after a latent period of 8 to 10 hours. After reviewing about 700 case histories, Balint (1974) observed the following signs and symptoms. Nausea, headache, general malaise, somnolence, loss of consciousness, convulsions, bloody diarrhea with tenesmus, dehydration, thirst, cyanosis, tachycardia, fall in blood pressure, changes in electrocardiogram, asthmatic symptoms, exanthema, liver necrosis, nephritis, proteinuria, rise in excretion of non-protein nitrogen, conjunctivitis, optic nerve lesion, mydriasis, leukocytoses and changes in biological data. At post mortem the main

changes noticed were bleeding in the serous membranes, hemorrhage in the stomach and intestines, degenerative changes in the heart as well as liver and kidneys, infiltrations of the lymph nodes and changes in the spleen, especially in its lymphoid elements. Specific references for each case are listed in the 1974 Balint paper.

In cases where a sub-lethal dose was administered the patient recovers with no lasting side effects (Crompton and Gall, 1980).

6. Detection of Ricin

The first method developed for ricin detection (Clarke, 1953) used anti-ricin antibodies. Methods developed since then include immunoassays, enzyme-linked immunosorbent assays (ELISA), radioimmunoassays (RIA), and immunocytochemical assays (Koja *et al.*, 1980, Godal *et al.*, 1983, Kopferschmitt *et al.*, 1983, Cmiech *et al.*, 1985; Griffiths *et al.*, 1986a, b, 1989a, b, Leith *et al.*, 1988).

Wannemaker *et al.* (1992) have published an article on ricin detection in castor bean extracts. They reviewed the methods developed for ricin assay, and reported the results of comparisons of various methods carried out in their laboratories. The results of their assays of the ricin content in a castor bean meal extract showed good agreement among the methods. The ricin content was found to be as follows: Mouse bioassay, from the median effective dose, 4.1 mg/ml; mouse bioassay, from the mean time to death, 3.7 mg/ml; Vero cell cytotoxicity, 4.9 mg/ml; ELISA, 1.8 mg/ml; HPLC, 3.3 mg/ml; SDS-PAGE, 2.9 mg/ml; and capillary electrophoresis, 3.3 mg/ml.

7. Treatment of Ricin Intoxication

No specific chemoprotective agents are available against ricin, therefore the only treatment available for ricin intoxication is symptomatic treatment. However, studies are ongoing to screen numerous compounds for their ability to alter ricin toxicity *in vitro* and ricin lethality *in vivo*. Brefeldin A, an antiviral macrolide antibiotic, inhibits the transport of proteins from the endoplasmic reticulum to the Golgi and causes disassembly of the Golgi stacks. Yoshida *et al.* (1989) found that pretreatment with brefeldin A reduced ricin-induced protein synthesis inhibition in Vero cells and in a ricin-sensitive mutant CHO (Chinese Hamster Ovary) cell line. Brefeldin A and 3'-azido-3'-deoxythymidine (AZT) were found to reduce ricin-induced protein

synthesis inhibition by 50% in cell culture, but not in a cell-free rabbit reticulocyte lysate system (Thompson and Pace, 1992). However, brefeldin A had no effect on ricin induced lethality when tested in a lethal mouse model (Wannemacher *et al.*, 1991a). Brefeldin has also been tested in an isolated perfused rat liver model and found to be minimally protective against ricin intoxication (Pace *et al.*, 1992). The studies suggest that brefeldin alters the bioavailability of ricin.

Indomethacin, a non-steroidal anti-inflammatory agent, decreased ricin-induced protein synthesis inhibition in cultured macrophages by decreasing the binding and internalization of ricin. However, fluocinolone, an anti-inflammatory glucocorticoid, increased ricin-induced protein synthesis inhibition by increasing binding and internalization of ricin (Naseem and Pace, 1992).

Lactose and lactulose decrease ricin binding to cell surface receptors and increase the rate of dissociation from receptors and thereby decrease ricin-induced protein synthesis inhibition in cell culture. However, pretreatment with lactose or lactulose had no effect on survival rate or mean time to death after an i.v. challenge with ricin (Wannemacher *et al.*, 1991b).

Mice can be protected from ricin lethality due to aerosol exposure by vaccination with ricin or by passive treatment with heterologous antibody (Hewetson *et al.*, 1991). This vaccination did not protect the mice from the pulmonary necrosis caused by aerosol ricin exposure.

8. Therapeutic Uses

Anti-tumor properties of ricin were recognized even before its mechanism of action was known. As early as 1951 Mosinger reported an effect of ricin on sarcomas in rats. In 1970, Lin *et al.* reported that mice could be cured of Ehrlich ascites tumors by an injection of ricin up to 5 days after intraperitoneal injection of 2×10^7 Ehrlich ascites tumor cells. Fodstad and Pihl in 1978 tested the effect of ricin and abrin (another toxic lectin) against 5-fluorouracil and doxorubicin (Adriamycin) in mice inoculated with L1210 leukemia cells. In mice injected intraperitoneally, ricin was superior to abrin and 5-fluorouracil but inferior to doxorubicin. This enhancement of survival was accomplished without the bone marrow depression that is caused by other cytotoxic agents. Olsnes and Refsnes (1978) found that HeLa cells are equally sensitive in all phases of the cell cycle. Thus, the reason leukemic

cells are more sensitive to ricin than normal bone marrow cells is still unclear. In mice injected intravenously with the leukemia cells, ricin failed to enhance survival. This is to be expected since the toxic lectin is unable to pass the blood-brain barrier. In 1980, Fodstad and Pihl tested the effect of ricin and doxorubicin together on mice inoculated with leukemia L1210 cells. They found a synergistic effect with the combination increasing life span by 198%. In 1984 Fodstad *et al.* carried out a phase I study of ricin in patients with solid cancers or malignant lymphomas who were no longer candidates for conventional therapy. Ricin was given in doses calculated to be one-third the "toxic dose low" in dogs, and side effects, pharmacokinetic parameters, antibody formation and antitumor effect were monitored. Most patients experienced fever, flu-like symptoms, fatigue and muscular pain. Some also experienced nausea and vomiting. It was determined that ricin is eliminated from the plasma according to first order kinetics and the half-life is dose dependent. Anti-ricin antibodies were detected 3 to 5 weeks after the first injection and increased with time. Of the 38 patients which were evaluated for antitumor effect, 1 had a partial response and 8 patients had stable disease.

The advent of monoclonal antibodies opened up a whole new world of possibilities for the therapeutic use of ricin. By targeting the toxin to a specific antigen and cell, the generalized toxic effect of ricin can be eliminated. An immunotoxin is created when Ricin A chain is reversibly coupled to an antibody which is targeted to a specific cell type. In 1982 Krolick *et al.* used an anti-immunoglobulin(Ig)-ricin A chain conjugate to rid the bone marrow of mice of murine B cell tumor cells before autologous bone marrow transplantation. In 1984 Colombatti *et al.* used Thy 1.2 monoclonal antibody-ricin A chain conjugate to rid the bone marrow cells of EL4 tumor cells. It was determined that the conjugate was effective in eliminating the tumor cells without adversely affecting the progenitor cells of the normal bone marrow. This conjugate would have a possible use in autologous bone marrow transplantation. Raso *et al.* (1982) used the ricin A chain conjugated to a monoclonal murine antibody specific for common acute lymphoblastic leukemia cell. The conjugate was an effective cytotoxic agent *in vitro* producing a 50% inhibition of proliferation at levels of 2×10^{-10}M for antigen positive cells while antigen negative cell lines remained unaffected up to a concentration of 10^{-6}M. Seto *et al.* (1982) investigated the *in vitro* and *in vivo* efficacy of ricin A chain conjugated to a monoclonal IgG2b antibody against mouse mammary tumors. Two of the five conjugate treated animals survived tumor free and the remaining animals exhibited an

increased life span 25% above that of control animals.

In 1984 Oladapo *et al.* investigated the cytotoxic effect of a monoclonal antibody to hepatitis B surface antigen conjugated to the intact ricin molecule. The antibody was attached to the B chain portion of ricin by the use of a mixed anhydride derivative of chlorambucil as the coupling agent. The conjugate was compared to native ricin in cytotoxic effect against Alexander primary liver cell carcinoma tumors in athymic mice. The conjugate was determined to be superior to native ricin in reducing tumor size. The *in vitro* efficacy of a monoclonal antibody-ricin A chain conjugate against human T cell leukemia/lymphoma virus type 1 (HTLV-1) was investigated by Krönke *et al.* (1985). They determined that the conjugate was able to eliminate >99.9% of the HTLV-1 infected T cell population at concentrations only marginally affecting the antigen negative cells. The specificity of this conjugate raises the possibility of the therapeutic use of the immunotoxin in HTLV-1 disease.

A significant problem arose when using the immunoconjugates *in vivo*. The mannose and fucose residues present on the ricin A chain were being recognized by receptors on the non-parenchymal and parenchymal cells of the liver which resulted in rapid clearance of the conjugate from the bloodstream (Blakey *et al.*, 1987 and Bourrie *et al.*, 1986). This problem can be alleviated by treating the toxin with a mixture of sodium metaperiodate and sodium cyanoborohydride which chemically modifies the carbohydrate in the molecule (Thorpe *et al.*, 1985). These deglycosylated conjugates are not taken up by liver cells and as a result are cleared from the bloodstream much less rapidly than the unmodified ricin immunoconjugates. In 1988 Kanellos *et al.* used the same modifying technique on native ricin and attached the whole ricin molecule to a monoclonal antibody. This treatment blocked the galactose binding sites on the B chain. They found not only an increased clearance time for the conjugate, but a high potency with high specificity. In 1989 Kanellos *et al.* tested the whole ricin-antibody conjugate in mice containing solid tumors. The conjugate was found to be effective in mice carrying thymoma grafts and in nude mice bearing human tumor xenografts. The thymomas and HT-29 tumors in nude mice completely regressed following an injection of the immunotoxin into the tumor. The tumors disappeared within 48 hours and in 80 and 100%, respectively, of the animals there was no recurrence. These results indicate that whole ricin-antibody conjugates have potential therapeutic use for local therapy, leading to the eradication of solid tumors by direct injection into the tumor.

In 1988 Griffin *et al.* investigated the *in vitro* cytotoxicity of a ricin A chain immunoconjugate against human adenocarcinomas of the colon and pancreas. An A chain derived from recombinant DNA procedures was used to eliminate any non-specificity due to contaminating B chain. A carboxylic ionophore, monensin, was used in an attempt to facilitate the entry of the immunotoxin into the cytosol. They determined that the immunotoxin was effective at low concentrations and monensin did potentiate the effect by a factor of 1.5 to 12.

In 1988 Press *et al.* compared three A chain immunotoxins directed against different epitopes on the CD2 molecule of malignant T cells. They concluded that all three had similar binding avidities and A chain activities; yet one immunotoxin was 100 - 1000 fold more effective in killing the malignant T cells. The immunotoxins were rapidly internalized, but their intracellular fates differed. The more toxic conjugate was retained for longer periods of time inside the cells and was more slowly degraded. The less effective conjugates were rapidly transported to lysosomes, digested and expelled. These results indicate that the epitope chosen for targeting by the monoclonal antibody may have a significant effect on the ability of the immunotoxin to translocate to the cytosol and kill the cells.

In 1990a Engert *et al.* evaluated deglycosylated ricin A chain immunotoxins directed against the CD30 antigen as potential reagents for the treatment of Hodgkin's disease. In the first study an *in vitro* assessment compared five monoclonal CD30 antibodies and two Fab' fragments each linked to deglycosylated ricin A chain. The smaller molecular weight Fab' fragments were tried in an effort to increase access of the immunotoxin to solid tumors. It was determined that ricin A chain immunotoxins constructed from the CD30 antibodies HRS-3, HRS-4, and Ber-H2 and their Fab' fragments are powerfully and specifically toxic to Hodgkin's cells *in vitro*. One of the antibodies (HRS-4) showed an unexpected cross-reactivity with normal and malignant pancreatic cells. The immunotoxin of choice for *in vivo* experimentation seemed to be the HRS-3-ricin A chain since it combined the highest *in vitro* potency and the least cross-reactivity with other tissues. A second study by Engert *et al.* (1990b) compared the *in vivo* efficacy of three monoclonal antibodies and two corresponding Fab' fragments attached to ricin A chain on solid human Hodgkin's tumors in mice. The Fab' fragments were 7.8 and 60 fold less cytotoxic than their intact counterparts. The effectiveness of the immunotoxins depended on the size of the tumor at the time of injection, since one of the immunotoxins (IRac-ricin A) induced

complete remissions in 100% of the animals with small tumors (10-20mm^3), but only 13% of mice with larger tumors (400-600mm^3). A single i.v. injection of immunotoxin corresponding to 40% of the LD_{50} induced lasting complete remissions in 38 to 50% of the animals with tumors of 60 to 80 mm^3 size. It was concluded that two of the immunotoxins (HRS-3-ricin A and IRac-ricin A) and the HRS-3 Fab'-ricin A were effective enough to be candidates for the treatment of Hodgkin's disease in humans.

Weiner *et al.* (1989) conducted a phase I evaluation of an anti-breast carcinoma monoclonal antibody-recombinant ricin A chain immunoconjugate. Four women with metastatic breast cancer were treated with the immunoconjugate (260F9-ricin A) and evaluated for side effects and effect on the disease. One patient had a clinical response, with the disappearance of her sole site of disease, a lung nodule. However, the tumor recurred at a chest wall/skin site 3.5 months later. Toxicities observed were malaise, fever, myalgias, anemia, weight gain, edema hypoalbuminemia, hypoproteinemia and eosinophilia. In addition, one patient experienced a nonpruritic rash on her trunk and extremities. All four patients developed anticonjugate antibody titers. They concluded that the toxic syndrome and the development of anticonjugate antibodies limited the likelihood of reaching therapeutically useful dosages with this immunoconjugate.

A phase I-II study was undertaken by Byers *et al.* (1990) to investigate the use of a ricin A chain immunotoxin in steroid-resistant acute graft-versus-host disease (AGVHD). The T-lymphocyte-mediated condition was treated using a monoclonal antibody (H65) that recognizes the CD5 lymphocyte differentiation antigen. The safety and efficacy of the immunoconjugate was evaluated in 34 patients with severe AGVHD. The principal side effects were constitutional symptoms such as fatigue and myalgias; hypoalbuminemia with weight gain was seen at all doses. Durable complete or partial responses were not dose-related and were seen in 16 patients. Skin GVHD had the highest incidence of response (73%), although often gastrointestinal tract GVHD (45%) and liver GVHD (28%) also showed improvement or resolution. Survival in responding patients was significantly prolonged at all times as compared with non-responders. The results of this study indicate that anti-T-lymphocyte immunotoxins may form a new class of immunosuppressive agents useful in T-lymphocyte-mediated diseases. One company (Xoma Corporation) has filed an NDA on a ricin immunotoxin targeted to an antigen on the T lymphocytes. This immunctoxin (XomaZyme®) would have an indication for the treatment of GHVD.

A variety of monoclonal antibodies have been used with ricin to create immunotoxins. LeMaistre *et al.* (1987) tested the murine monoclonal antibody $323/A_3$ attached to the A chain of ricin as a treatment for breast cancer cells *in vitro*. Ricin antibodies against human ovarian cancer were tested by Ettenson *et al.* (1988) and FitzGerald *et al.* (1987) in cell culture and in a mouse model, respectively. Marks *et al.* (1990) tested the efficacy of ricin immunotoxins potentiated with monensin against ovarian adenocarcinoma in cell culture and a mouse model. Roth *et al.* (1988) used ricin immunotoxins targeted to fibroblasts transformed with the Kirsten sarcoma in attempt to cure pulmonary metastases in mice. In 1987 (Griffin *et al.*) the antitumor activity of ricin immunotoxin in a mouse model of human malignant mesothelioma was evaluated. In 1987 patients with stage III metastatic malignant melanoma were treated with ricin (Spitler *et al.*). The cytotoxic effect of a ricin immunotoxin targeted to human malignant brain tumor cells was evaluated by Zovickian *et al.* in 1987. Zenner (1986) studied the selective killing of laryngeal carcinoma cells by a ricin immunotoxin. Several leukemias have been the focus of study using ricin immunotoxins. Gregg *et al.* (1987) studied both whole ricin and ricin A chain immunotoxins for therapy of guinea pig L_2C B cell leukemia. Fulton *et al.* (1988) tested ricin A chain immunotoxins as therapy for BCL_1 tumors in mice. A Phase I study of T101-ricin A chain immunotoxin in refractory chronic lymphocytic leukemia was undertaken in 1988 (Hertler *et al.*). The results of these studies varied from no sustained benefit (in a Phase I study, Hertler *et al.*, 1988) to a 90% reduction in tumor size (in a mouse model, Fulton *et al.*, 1988). The *in vitro* studies indicated that some immunotoxins merited further studies while others were ineffective. These studies demonstrate that the choice of target epitope on the antigen may determine the efficacy of the immunotoxin.

Rheumatoid arthritis is an autoimmune disease characterized by inflammation of the synovial membrane of joints. In rheumatoid arthritis, the synovium contains increased numbers of activated lymphocytes more than 80% of which are T cells (Janossy *et al.*, 1981). Removal of T cells by draining of the thoracic duct has resulted in clinical improvement for the arthritic patient (Paulus *et al.*, 1977). It may be possible to effectively treat rheumatoid arthritis by attacking the $CD4^+$ T cells with an immunotoxin. It may be possible to raise monoclonal antibodies to the T cell receptor on the $CD4^+$ T cells of rheumatoid arthritis patients and use these to target ricin A chain immunotoxins specifically to the T cells involved in rheumatoid arthritis (Blakey and Thorpe, 1988).

The retrograde axoplasmic transport of ricin has been taken advantage of in an attempt to treat latent Herpes Simplex virus in the mouse (Hino *et al.*, 1988). Mice were inoculated with HSV-1 F strain by subcutaneous injection in the lip. Two to nine months later, ricin was injected at the same site. Ricin was effective at eliminating the latent virus in the subregion of the ganglion corresponding to the injection site, but did not kill the latent virus that had spread to distant areas of the ganglion.

The treatment of AIDS by attacking the Human Immunodeficiency Virus (HIV) is another possible therapeutic use for ricin immunotoxins. The HIV envelope glycoprotein, gp120 is expressed on the surface of many HIV-infected cells and binds to CD4, a T helper cell surface molecule. In 1988 Till *et al.* used a soluble recombinant CD4-ricin A chain conjugate to kill HIV-infected H9 cells *in vitro*. The conjugate was not toxic to uninfected H9 cells and were not toxic to Daudi cells which express MHC class II antigens. This immunotoxin is particularly attractive because the CD4 binding site is the most highly conserved region among different strains of HIV. In 1989 Pincus *et al.* tested a monoclonal antibody-ricin A chain conjugate targeted to the viral envelope protein $gp120^2$ on cells infected with the LAV/HTLV-IIIB strain of HIV. This glycoprotein is expressed on the cell surface during active production of the virus. The immunoconjugate inhibited protein synthesis and cell growth in HIV-infected cells and most importantly, markedly inhibited the production of infectious virus. Another antibody (BM-1) which recognizes a carbohydrate antigen on the surface of virally infected cells was conjugated to ricin A chain. This immunoconjugate did not kill HIV-infected cells, emphasizing the importance of the target antigen in the efficacy of the immunotoxin. These studies indicate that ricin-antibody conjugates may have a future role in the treatment of AIDS.

In summary, ricin is a toxic glycoprotein lectin which enters the cell and inhibits protein synthesis by inactivating the 28S ribosomal subunit. Antibody-ricin immunotoxins have been used to selectiveiy kill unwanted cells in experimental disease models and in clinical studies. Ricin immunotoxins have potential therapeutic use in the treatment of several diseases including cancers, rheumatoid arthritis, and viral infections such as those caused by the herpes simplex and human immunodeficiency viruses.

C. Purpose of the Present Work

This study is designed to supply information on the effects of ricin on

the cardiovascular system, including its lethality in rabbits, its effects on blood flow, ECG, heart rate, and blood pressure, as well as its actions at the level of the nerve, smooth muscle cells and enzymes of the vascular system.

This information should be important for designing more effective therapy for ricin intoxication.

D. Methods of Approach

In order to establish doses to be used in these studies, the LD_{50} of ricin administered i.v. to male rabbits was determined by the Up and Down Method (Dixon, 1965). From these studies, we determined a minimum lethal dose and a toxic sub-lethal dose. We investigated histopathology following ricin in rabbits used in the lethality study. With these doses determined, further studies were done on the cardiovascular effects of the minimum lethal dose and a toxic sub-lethal dose. The first study was a determination of the effects of these ricin doses on blood pressure, heart rate, and ECG from the period 12 hours after ricin injection for the next 36 consecutive hours, with determinations being made every hour. Following this, the same experiment was done on the first 12 hours after ricin administration. The effects of these two doses of ricin on blood flow were then studied at two intervals after ricin injection using the technique of radioactive microspheres.

In other studies, we determined the effects of ricin on the vascular neuro-effector system. These included effects of ricin on the ability of vascular preparations to contract and to relax, and determination of the effects of ricin on the vascular norepinephrine (NE) content, and the uptake and release of NE by blood vessels. We also studied the effect of ricin on NE metabolism by examining its effect on monoamine oxidase and catechol-*O*-methyl transferase activity.

We also studied the uptake and release of calcium by vascular tissue.

II. EXPERIMENTAL METHODS

A. Determination of the LD_{50}, Minimum Lethal and Toxic Sub-lethal Doses of Ricin in Male New Zealand White Rabbits

The LD_{50} of ricin in male New Zealand White rabbits ranging in weight from 1.64 to 2.39 kg was determined by a modified up-and-down method (Dixon, 1965). The steps in an experiment using the modified up-and-down procedure are as follows: a) A series of test levels is chosen with equal spacing between doses. b) A series of trials is carried out increasing the dose following a non-response (the animal is alive at a predetermined time point following toxin injection) and decreasing the dose following a response (death). c) Testing continues until a chosen "nominal" sample size (N) is reached. N is the total number of trials reduced by one less than the number of like responses at the beginning of the series. d) The resulting configuration of responses and non-responses for each series and the N is referred to the table of maximum likelihood solutions for the LD_{50} to obtain the value of k. One computes the LD_{50} from the following formula: $X_f + kd = LD_{50}$ where X_f is the last dose administered, k is the value from the table and d is the interval between doses. For this determination of LD_{50}, survival at 48 hours after an i.v. injection of ricin into the marginal ear vein was monitored. The initial dose was 0.439 μg/kg and the dose interval was 0.114 μg/kg. The minimum lethal dose was set as the lowest dose which resulted in the death of a rabbit during the LD_{50} determination. The toxic sub-lethal dose was set at one-half the minimum lethal dose.

B. Effects of the Minimum Lethal and Toxic Sub-lethal Doses of Ricin on Rabbit Blood Pressure, Heart Rate, and ECG Patterns

1. Observations for Hours 12 Through 48

After preparation of the rabbit for blood pressure and ECG determinations as described below, rabbits were placed into restraining boxes from which their heads protruded. They remained in the boxes for determination of blood pressure and ECG, and were removed from the boxes for approximately one-half hour about every three hours. During this half hour period they had unlimited access to food and water. Rabbits were given either a toxic sub-lethal dose or a minimum lethal dose of ricin, or a sham injection as a control. Blood pressure and ECG were determined every hour

throughout the time period.

a. Blood Pressure

Hair was removed from the rabbit tail with clippers. Blood pressures were determined by use of a tail cuff and sensor, and a Model 29 pulse amplifier (IITC, Inc., Woodland Hills, CA). Systolic pressures were read and diastolic values calculated from the printouts. Three consecutive blood pressures were obtained each hour, at approximately two minute intervals. These were averaged to obtain the blood pressure.

b. ECG

Hair was removed with clippers from an area approximately 4 cm by 4 cm on either side of the rib cage and on the middle posterior of the back of a rabbit. Electrodes were inserted subcutaneously within these areas for approximately 6 mm, then exteriorized and fastened. (The electrodes were made of sharpened 29 gauge stainless steel wire bent to somewhat resemble safety-pins.) Small alligator clip leads connected these to the Narco recording equipment. The electrodes were left in place for the duration of the experiment, while the alligator clip leads were removed when the rabbit was removed from the restraining box. The ECG was printed for 15 seconds every hour during the experiment.

c. Heart Rate

Heart rate was determined from the ECG tracings.

2. Observations for Hours 0 Through 12, (on Another Group of Rabbits)

a. Blood Pressure

Blood pressure was determined prior to injection and for the first twelve hours following injection of a toxic sub-lethal dose or a minimum lethal dose of ricin as in the study on hours 12 to 48, on a different group of rabbits. Sham-injected rabbits served as a control group.

C. Pathological Changes Following Ricin Injection in Rabbits

Rabbits were given 0.57 μg/kg (106% of the LD_{50}). Those that died had tissues removed for histopathological examination within two hours after death. Tissues were fixed in 4% formaldehyde, then embedded in paraffin, stained with hematoxylin and eosin, and examined under a microscope. Tissues examined included the liver, lungs, thymus, stomach, heart, kidney, urinary bladder, spleen, brain, adrenals, small intestine, cecum, colon, esophagus, gall bladder, lymph nodes, testicles, pancreas and salivary glands.

D. Alterations in Laboratory Values Following Administration of Ricin to Male Rabbits

Blood was drawn from the central ear artery before ricin administration and at 12, 24, 36, and 48 hours after ricin administration. Serum analyses were done by Vet Path Laboratories, Sapulpa, OK, by use of a Hitachi 705 Multichannel Analyzer, a totally automated system. Analyses included lactate dehydrogenase, creatine phosphokinase, blood urea nitrogen, calcium, glucose, phosphorus, total protein, albumin, globulin, albumin/globulin ratio; cholesterol, total bilirubin, alkaline phosphatase, serum glutamic pyruvic transaminase, gamma glutamyl transpeptidase, creatinine, amylase, sodium, and potassium.

E. Alterations in Laboratory Values and Lethality Following Administration of Ricin to Older Female Rabbits

Blood was obtained from older female rabbits, ranging in weights from 2.67 to 3.41 kg following ricin administration. Blood was drawn and serum analyzed as in the studies in male rabbits.

F. Effects of Ricin Administration on Blood Flow and Blood Flow Distribution Using Radio-labeled Microspheres

1. Surgical techniques

Twelve New Zealand White rabbits were studied. The animals, weighing between 1.8 and 2.2 kg, were anesthetized with intravenous sodium

pentobarbital (30 mg/kg). The submandibular region together with the left medial thigh and flank were shaved and sterilized with tincture of iodine and 70% ethanol. A small vertical incision, about 2.5 cm long, was made anteriorly in the right side of the neck and the right common carotid artery was exposed. A polyethylene catheter (i.d. 0.58 mm, o.d. 0.96 mm) was introduced into the right common carotid artery, advanced some 6-8 cm into the left ventricle and secured with 3/0 sutures. The catheter exited at the dorsum of the neck through a subcutaneous tunnel. Another catheter was introduced into the abdominal aorta through the femoral artery. Both catheters were imbedded subcutaneously. Radioactive microspheres were injected 24 hours after surgery.

2. Experimental procedures

Two rabbits were used as control animals. They were each given a sham injection of 1 ml of normal saline 12 hours before injection of the microspheres. The rest of the rabbits were divided into a high dose group receiving a minimum lethal dose of ricin and a low dose group receiving a toxic sub-lethal dose of ricin. In each group, rabbits were given ricin 12 or 18 hours before microsphere injection. Injection of microspheres was 24 hours after surgery.

Before blood flow determinations, the rabbit was fasted 24 hours in order to let the GI tract empty. The animal was kept calm during the experiment. About 800,000 microspheres (15 μ NEN-Trac microspheres, 40 mCi of Chromium-51 per gm, Dupont Chemical Co.) in 0.5 ml saline solution from a well-shaken vial were mixed with 1.5 ml of blood, sonicated and warmed to a temperature close to that of the rabbit. Then the mixture was slowly injected into the left ventricle. For ten seconds before and for one minute after the injection, a blood sample was withdrawn at 2.16 ml/min by a withdrawal pump (Harvard Apparatus). This served as a surrogate organ.

Two minutes after microsphere injection, the rabbit was sacrificed by injection of excess sodium pentobarbital and tissue samples were obtained. The skin, heart, aorta, lungs, trachea, bronchial tree, fat, liver, gall bladder, spleen, kidneys, adrenals, muscle, testes, brain, esophagus, stomach, and intestines were removed and weighed. Some organs were divided into subsections. For instance with the heart, inner, middle, and outer tissue samples were taken. For small organs, the total counts were determined by

putting them in more than one counting vial. For larger organs, several pieces of tissue were counted. Counts were averaged and extrapolated to obtain the total counts for the whole organs. Three pieces of skin were taken from the leg, abdomen and chest. The muscle sample was taken from the gluteus maximus muscle. All samples were packed to the same height in the counting tubes to minimize differences in counting efficiency.

3. The radioactive microsphere technique

Chromium-51 labeled 15 μm diameter microspheres were prepared for injection. The total amount of radioactivity of the microspheres was determined using a radioisotope dose calibrator CRC-10R (Capintec). Before injection, the mixture was warmed and well sonicated to keep the microspheres evenly distributed. The injection time length was kept at about 40 seconds. The catheter was flushed with saline to ensure that all microspheres had been injected and clamped to prevent fluid leaking back. The syringe together with the attached needle was removed and counted in the dose calibrator. The net total radioactivity injected was calculated by subtracting the radioactivity remaining in the syringe from the original radioactivity. All tissue samples, reference blood samples and both catheters were counted using a gamma counter (Multi-Prias Gamma Counting System, Model A5302, Packard Instrument Co). The relationship between the radioactivity measured in the dose calibrator and the gamma counter (CPM) was calculated by counting several standard samples in both. The cardiac output and tissue blood flow were calculated according to Hale's equation (Hales, 1974):

$$\text{Cardiac Output} = F_a \cdot I_{total} / I_a$$

$$\text{Organ Blood Flow} = F_a \cdot I_{tissue} / I_a$$

Where F_a = reference sample withdrawal rate.

I_{total} = total dose of radioactivity injected in μCi.

I_a = amount of radioactivity in the reference sample μCi.

I_{tissue} = amount of radioactivity in each organ in μCi.

Muscle blood flow was calculated as blood flow per 100 g tissue.

4. Evaluation of the adequacy of the mixing of microspheres and blood in the left ventricle

Blood flow to the right and left kidneys were compared in order to determine the adequacy of mixing of microspheres and blood flowing past the renal arteries and therefore the adequacy of mixing of blood and microspheres in the left ventricle.

G. Experimental Design for Studying the Physiological and Biochemical Changes in Rabbit Tissues Following Ricin Administration

For these studies the rabbits were divided into seven groups of six rabbits each, a control group and six treatment groups. The rabbits in the control group were given an i.v. sham injection and the rabbits in the treatment groups were given a minimum lethal dose or toxic sub-lethal dose of ricin i.v. and either 18 hours, 4 days or 7 days post injection, the rabbits were euthanized by an i.v. injection of pentobarbital and exsanguinated. The tissues were quickly removed and either placed in aerated Krebs solution for use that day, or rinsed in ice-cold saline, blotted and then frozen at -70°C for enzyme or norepinephrine content determination at a later time. Blood samples were obtained and centrifuged at 1000 g for 10 minutes to obtain the plasma. The plasma was frozen at -70°C for later determination of norepinephrine content.

H. Effects of Ricin Administration to Rabbits on Contractions and Relaxations of the Helically-Cut Central Ear Artery to Agonists

For the studies in which tension was monitored, the central ear artery from a control rabbit or from one of the six ricin treated groups was removed quickly, placed into aerated Krebs solution, cleaned of adhering tissue, and helically cut under a dissecting microscope into 3 strips approximately 1 mm by 13 mm.

Each strip was tied at both ends and suspended under 1 g tension in Krebs solution at a pH of 7.4 in an isolated muscle bath maintained at 38°C.

The Krebs solution was aerated with 95% O_2-5% CO_2 for at least 30 minutes prior to use and continuously while in the muscle bath. Tension was measured using Metrigram isometric force transducers (Model 797159-1, Gould Inc, Cleveland OH) and recorded by a Gould RS 3800 recorder.

Before all experiments, the strips were equilibrated under tension for 90 minutes, during which the bathing fluid was changed every 15 minutes. After the equilibration period, 120 mM KCl was added to the baths and the contraction recorded. The muscle was allowed to rest for 60 minutes before a second contraction to KCl was obtained. Two contractions to 0.1 mM tyramine were then obtained at 60 minute intervals. The bathing fluid was changed every 15 minutes between contractions. Then 1 nM to 0.1 mM norepinephrine was added in a cumulative fashion in half log increments. When the tissue was maximally contracted 0.1 mM papaverine was added to relax the artery. The contractions to agonists and the relaxations to papaverine are reported as g tension/mm^2 of tissue.

I. Effects of Ricin Administration on the Relaxation of Norepinephrine-Contracted Aorta Rings to Relaxant Compounds

For the studies in which tension was monitored, the upper one-half of the thoracic aorta was removed quickly, placed into aerated Krebs solution, cleaned of adhering tissue, and then cut into rings approximately 3 mm wide.

Each ring was suspended under 2 g tension in Krebs solution at a pH of 7.4 in an isolated muscle bath maintained at 38°C.. The Krebs solution was aerated with 95% O_2-5% CO_2 for at least 30 minutes prior to use and continuously while in the muscle bath. Tension was measured using isometric transducers and recorded by a Gould recorder.

Before all experiments, the rings were equilibrated under tension for 90 minutes, and the bathing fluid was changed every 15 minutes. After the equilibration period 120 mM KCl was added to the baths and the contraction recorded. Repeated exposures to a submaximal concentration of norepinephrine were made until contractions obtained were consistent. Ten minutes prior to all subsequent norepinephrine contractions 1 μM propranolol, to block β receptors, and 1 μM imipramine, to block amine uptake, were added to the baths. Then the rings were contracted with 1 μM norepinephrine and

when a steady state contraction was obtained, 1 nM to 0.1 mM methacholine was added in a cumulative fashion and the relaxations recorded. The artery was allowed to rest for 60 minutes with the bathing fluid changed every 15 minutes. The artery was again contracted with 1 μM norepinephrine and when a steady state contraction had been reached 1 μM ATP was added and the relaxation recorded. After another 60 minute rest period, the artery was again contracted with 1 μM norepinephrine and when a steady state contraction had been reached, 1 nM to 0.1 mM papaverine was added in a cumulative fashion and the relaxations recorded. The relaxations are reported as percent of initial norepinephrine contraction.

J. Effects of Ricin Administration to Rabbits on the Norepinephrine Content of Their Thoracic Aortas and Plasma

Norepinephrine content was measured by an electro-chemical detector following high performance liquid chromatography (HPLC) based on the methods of Griffith *et al.* (1982) and Keller, *et al.,* (1976).

The aorta was homogenized in 0.1 M chilled perchloric acid ($HClO_4$) using a Brinkmann Polytron homogenizer and centrifuged at 1000 g for 20 minutes at 5°C. A 200 μl aliquot of the supernatant solution or 1 ml of plasma was placed on 10 mg of acid-washed alumina, along with 50 μl of solution containing 100 ng/ml of the internal standard, 3,4-dihydroxybenzylamine (DHBA). The pH was adjusted to 8.6 by the addition of 1 ml of 1.5 M Tris buffer. The mixture was shaken on a reciprocating shaker for 10 minutes and the alumina was allowed to settle. The liquid was removed and the alumina was washed twice with 2 ml of water and aspirated to near-dryness. The alumina was mixed with 200 μl of 0.1 M $HClO_4$, then removed by placing it in a microfilter-containing centrifuge tube and centrifuging it at 1000 g for 1 minute. Fifty microliters of the filtrate was injected into the HPLC. (This amount totally fills the 20 μl loop with the excess spilling out).

The HPLC was operated at 35°C with a flow rate of 1.5 ml/minute. The column was a BAS MF 6017, containing Biophase ODS, 5μ C-18 spherical particles. The electro-chemical detector was set at an electrical potential of 750 mvolts. The mobile phase was prepared by dissolving 28.3 g of monochloroacetic acid (MCAA), 9.35 g of sodium hydroxide, 1.5 g of ethylene diamine tetraacetic acid, and 25-30 mg of sodium octyl sulfate (SOS)

in 2 liters of distilled water. The pH was adjusted to 3.00 to 3.05 with solid MCAA or NaOH as required. The mobile phase was filtered through a 0.2 μ pore filter and degassed by stirring in a partial vacuum before use.

The norepinephrine content of the samples was calculated by comparison of the peak heights to the internal standard. Norepinephrine content is reported as μg/mg wet weight of aorta and ng/ml of plasma.

K. Effects of Ricin Administration to Rabbits on Norepinephrine Efflux From the Aorta During Transmural Nerve Stimulation

The aorta was removed from the rabbit and placed in Krebs solution. It was cleaned of adhering connective tissue and cut helically into two strips approximately 3 mm by 15 mm. These were hung under 2 g tension in two isolated muscle baths between platinum electrodes in Krebs solution at 38°C. The strips were then incubated for 90 minutes in 0.1 μM tracer labeled norepinephrine. After the incubation period the radioactive solution was drained and the strips washed twice with Krebs solution. A superfusion of Krebs solution at 38°C was begun at a rate of 4 ml/min. Two minute fractions of the superfusate were collected by a fraction collector. The electrodes were attached to a Grass S88 stimulator and at 12 minutes after the beginning of superfusion the muscle was stimulated for 90 seconds at a supramaximal stimulation of 40 volts of 0.3 ms duration at a frequency of 2 Hz. Sixteen minutes later the tissue was stimulated at 10 Hz with the other parameters remaining the same. At 16 minute intervals the tissue was again stimulated at 2 Hz and then again at 10 Hz. An aliquot of each 2 minute fraction of superfusate was placed in a 20 ml counting vial and 5 ml of scintillation fluid (Scintiverse BD, Fisher Scientific) was added. The radioactivity was counted by scintillation spectrometry (Beckman LS1801 Liquid Scintillation spectrometer). At the end of the experiment the aorta was placed in a 20 ml counting vial, 0.5 ml of Soluene was added and the aorta was allowed to dissolve overnight. Then 5 ml of scintillation fluid was added to the vial and the radioactivity in the tissue was determined. Counts per minute (CPM) were converted to disintegrations per minute (DPM) by the use of H# and counting efficiency. The fraction of norepinephrine released per pulse was determined by dividing the washout of radioactivity during the stimulation minus the background washout, by the total amount of radioactivity in the tissue at that time. The data are reported as the fraction of norepinephrine efflux per pulse. The norepinephrine efflux in

ng/mg of tissue/pulse was also calculated. This was done by multiplying the fraction of norepinephrine efflux by the amount of norepinephrine contained in the aorta of other rabbits from the same experimental group as determined by HPLC.

L. Effects of Ricin Administration to Rabbits on Norepinephrine Uptake by the Aorta

Aorta was removed from the rabbit and placed in aerated Krebs solution. It was cleaned of adhering connective tissue and cut helically into strips approximately 3 mm by 15 mm. It was then incubated for 90 minutes in tracer labeled 0.1 μM norepinephrine (5 μCi [^{3}H]-norepinephrine per strip). After the incubation period the radioactive solution was drained and the strip was rinsed twice with 4 ml of Krebs solution. Aliquots of the radioactive solution and each of the rinse solutions were placed into 20 ml counting vials and 5 ml of scintillation fluid was added. The strip was placed in a 20 ml counting vial and 0.5 ml of Soluene was added. The strip was allowed to dissolve overnight. Five mls of scintillation fluid was added and the radioactivity of all vials was determined using scintillation spectrometry. Counts per minute were converted to disintegrations per minute by the use of H# and counting efficiency. Norepinephrine uptake was calculated by dividing DPM in the tissue by total DPM (radioactive solution + two washes + tissue DPM) and multiplying this fraction by the total moles of norepinephrine in the incubation solution. The results are expressed as pM of norepinephrine/mg wet weight of tissue.

M. Effects of Ricin Administration to Rabbits on Monoamine Oxidase Activity of Various Tissues

The determination of monoamine oxidase was by the method of Wurtman and Axelrod (1963). Fifteen milligrams of tissue were homogenized in 1 ml of ice-cold 0.15 M KCl solution with a Brinkmann Polytron homogenizer at a setting of 3 for three 15 second bursts then centrifuged at 1,000 g for 20 minutes at 5°C. One hundred microliters of the supernatant were placed into a reaction tube which contained 100 μl of 0.5M phosphate buffer at pH 7.4, 50 μl of 1 mM tryptamine and 0.05 μCi of [^{3}H]-tryptamine. The tubes were agitated for 20 minutes at 38°C, then the reaction was stopped by the addition of 0.2 ml of 2 N HCl. The tryptamine metabolite

(indoleacetic acid) was extracted into 4 ml of toluene by mixing on a Vortex mixer for 30 seconds. The layers were separated by centrifuging for 5 minutes at 1000 g. A 2 ml aliquot was removed from the organic layer and the radioactivity from the tryptamine metabolite counted by scintillation spectrometry. Boiled enzyme and zero time blanks were used. Following conversion of CPM to DPM the greater of the two blank values was subtracted and a mathematical equation was used to convert the radioactivity extracted to moles of substrate deaminated. Data were expressed as nanomoles of substrate deaminated /g wet weight of tissue/minute.

N. Effects of Ricin Administration to Rabbits on Catechol-O-Methyltransferase Activity of the Aorta

Catechol-*O*-methyl transferase activity determination was based on the method of Axelrod (1962) as modified by Wrenn *et al.* (1979) and Roth (1980). Thirty to ninety milligrams of aorta were placed in 1 ml of ice-cold 0.05 M phosphate buffer at pH 7.4 and homogenized with a Brinkmann Polytron homogenizer at a setting of 3 for three 15 second bursts. The homogenates were then centrifuged at 43,000 g for 20 minutes at 5°C. Four hundred microliters of the supernatant were placed into a reaction tube which contained 300 μl of 0.05M phosphate buffer at pH 7.4, 12.5 μl of 0.1 M MgCl, 50 μl of 10 mM S-adenosylmethionine, 50 μl of 10 mM pargyline, 50 μl of 4 mM dopamine and 0.2 μCi of [^{3}H]-dopamine. The tubes were agitated for 40 minutes at 38°C, then the reaction was stopped by the addition of 1.5 ml of 0.5 M potassium borate buffer at pH 10.0. The *O*-methylated dopamine metabolite was extracted into 5 ml of toluene/isoamyl alcohol (3:2,V:V) by mixing on a Vortex mixer for 30 seconds. The layers were separated by centrifuging for 5 minutes at 1000 g. A 2 ml aliquot was removed from the organic layer and the radioactivity from the labeled dopamine metabolite was counted using scintillation spectrometry. Boiled enzyme and zero time blanks were used. After conversion of CPM to DPM the greater of the two blank values was subtracted and a mathematical equation was used to convert the radioactivity extracted to moles of substrate methylated. The data were expressed as nanomoles of substrate methylated/g wet weight of tissue/minute.

O. Effects of Ricin Administration to Rabbits on Cyclic-AMP Activity in the Plasma

Freshly drawn blood from the control or from rabbits given a minimum lethal dose of ricin was mixed with 1% of its volume of 0.5 M EDTA (pH 7.5) in a cooled centrifuge tube. The mixture was centrifuged and the plasma was collected for determination of its cAMP activity. EDTA was used to prevent degradation of cAMP because it inhibits phosphodiesterase. In addition, EDTA is also an anticoagulant. Cyclic AMP was determined using an assay kit from Amersham Corporation (Arlington Heights, IL). The method is based on the competition between unlabelled cAMP and a known amount of [^{3}H]-cAMP for binding to a cAMP-dependent protein kinase. Cyclic AMP concentrations are expressed as picomoles/ml of plasma.

P. Effects of Ricin Administration to Rabbits on Calcium Uptake by the Rabbit Aorta.

Aortas from control rabbits and rabbits given either a toxic sub-lethal dose or a minimum lethal dose of ricin were placed into physiological solution, cleaned of adhering tissues, and cut into strips approximately 3 mm wide. The adventitia was removed by the method of Maxwell (1968), and the endothelium was removed in the process.

Strips were equilibrated in physiological solution (pH 7.2) for 60 min at 37°C while aerating with a gas mixture of 95% O_2 and 5% CO_2. Tissues were incubated in physiological solution containing $^{45}Ca^{2+}$ (0.5 μCi/ml) for 10 min before adding 100 μM norepinephrine and leaving it in the solution for 2 min. The strips then were placed for 2 min in 10 ml of a lanthanum solution to remove extracellular Ca^{2+} from the tissue (Godfraind and Miller, 1982). Because lanthanum has a similar ionic radius to Ca^{2+} and a greater positive charge, it binds more tightly than Ca^{2+} at superficial Ca^{2+} sites (Weiss, 1974). The tissues were then blotted, weighed, and extracted with 3 ml of hypotonic EDTA solution (5 mM) overnight (Meisheri *et al.*, 1980). Seven ml of scintillation cocktail (ScintiVerse™) was added to each of the vials of EDTA solution and to a vial containing a 10 μl aliquot of the $^{45}Ca^{2+}$ solution. The vials were counted in a liquid scintillation counter.

In other experiments, tissues were incubated with physiological solution containing 80 mM KCl with reduced NaCl and $^{45}Ca^{2+}$ (0.5 μCi/ml) for 10 min, after the 60 min equilibration period. Strips were removed from the radioactive solution, placed into the lanthanum solution and handled as

described above.

For the time course experiments of $^{45}Ca^{2+}$ uptake, following the 60 min equilibration period, tissues were incubated in physiological solution containing $^{45}Ca^{2+}$ (0.5 μCi/ml) for 1, 3, 6, 10 or 15 min. Strips were removed from the radioactive solution, placed into the lanthanum solution and handled as described above.

$^{45}Ca^{2+}$ uptake was calculated based on the following (Rico *et al.*, 1990):

$^{45}Ca^{2+}$ (μmol/g wet weight) =
dpm in muscle x μmol Ca^{2+} in medium/dpm in medium x wet weight (g)

Q. Effects of Ricin Administration to Rabbits on Calcium Efflux by the Rabbit Aorta

Aorta strips were attached to stainless steel hooks and equilibrated in aerated physiological solution (pH 7.2) containing $^{45}Ca^{2+}$ (1 μCi/ml) for 3 h at 37° C. Each strip was then rinsed with 150 ml physiological solution for 5 sec and transferred at 5 min intervals through a series of ten vials each containing 3 ml physiological solution. The vials, into which they were placed into at 25 min contained 80 mM KCl (with the NaCl content of the physiological solution reduced an amount equimolar to the added KCl). The vials into which they were placed at 40 min contained 100 μM NE in physiological solution. At the end of the 50 min period, the strips were blotted, weighed and extracted with 3 ml of hypotonic EDTA solution (5 mM) overnight. Radioactivity in the EDTA solutions in the series of 10 vials was determined in a liquid scintillation counter after adding 7 ml of scintillation cocktail (ScintiVerse™) into those vials. The rate of $^{45}Ca^{2+}$ efflux was calculated based on the following (Rico *et al.*, 1990):

Rate of $^{45}Ca^{2+}$ efflux =
$^{45}Ca^{2+}$ (dpm) lost during TI/ $^{45}Ca^{2+}$ (dpm) remaining in strip x TI

TI = time interval in minutes

R. Drugs and Solutions

Drugs obtained from Sigma Chemical Co. (St. Louis, MO) include:

Ricin
Tyramine HCl
Papaverine HCl
Imipramine HCl
Propranolol HCl
Methacholine (acetyl β methylcholine)
Adenosine 5'-triphosphate
Dopamine HCl
Tryptamine HCl
Pargyline HCl
S-adenosyl methionine p-toluenesulfonate
Dihydroxybenzylamine HBr
Lanthanum Cl
EDTA disodium
Tris-hydroxymethylaminomethane

Drugs obtained from Du Pont Chemical Co. (Boston, MA) include:

NEN-Trac chromium-51 15μ microspheres, 40 mCi/g
l-[7-^{3}H]-Norepinephrine, 11.4 Ci/mmol
[Ethyl-^{3}H]-tryptamine HCl, 30 Ci/mmol
^{45}Ca, 20 mCi/ml

Drugs obtained from other companies include:

Pentobarbital sodium 64.8 mg/ml - Fort Dodge Lab Inc. (Ft. Dodge, KS)
l-Norepinephrine d-bitartrate - a gift from Sterling Winthrop (New York, NY)
Sodium octyl sulfate - Bioanalytical Systems (West Lafayette, IN)
Perchloric acid - J.T. Baker Chemical Co. (Phillipsburg, NJ)
[2,5,6-^{3}H]-Dopamine, 11.9 Ci/mmol - Amersham (Arlington Heights, IL)

Physiological solutions used contained the following:

Krebs physiological solution (in mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 1.6; KH_2PO_4, 1.2; $MgSO_4$, 0.7; $NaHCO_3$, 25; d-glucose, 11; ascorbic acid, 0.06; EDTA, 0.03; pH 7.4.

Calcium uptake and efflux physiological solution (in mM): NaCl, 122; $MgCl_2$, 0.38; KCl, 5.9; d-glucose, 11.5; $NaHCO_3$, 15.0; $CaCl_2$, 1.25.

Lanthanum solution (in mM): NaCl, 122; $MgCl_2$, 0.38; KCl, 5.9; D-glucose, 11.5; $LaCl_3$, 50; tris maleate, 15; pH 6.8.

Physiological solutions were made fresh daily using millipore-filtered distilled water. Stock solutions of drugs were made in distilled water and frozen at -20°C, with the exceptions of norepinephrine which was made in 0.1 N HCl and ATP which was made fresh daily within 30 minutes of use. Serial dilutions of stock solutions were made fresh daily in distilled water.

Ricin was purchased in a concentrated solution of 1.8 mg of protein/ml. It was diluted in 0.9% NaCl solution to yield a concentration of 10 μg/ml.

S. Statistical Methods

The results of all experiments except calcium efflux and uptake were tested for statistical significance using Analysis of Variance with Dunnett's test for comparison of multiple treatments to control. The calcium data was tested for statistical significance using Duncan's New Multiple Range Test. In this study, p values of 0.05 or less were considered significant.

III. RESULTS

A. The LD_{50} Minimum Lethal and Toxic Sub-lethal Doses of Ricin in Male New Zealand White Rabbits

As has been previously reported (Olsnes *et al.*, 1976) there was a lag period following ricin administration before signs of toxicity and lethality were observed with no signs of toxicity observed for 12 hours after ricin administration. All animals that died did so between 22 and 48 hours following ricin administration. We found, as have other investigators (Fodstad *et al.*, 1976), that the dose-response curve to ricin is quite steep. The 48 hour LD_{50} of ricin given i.v. in male New Zealand White rabbits determined by the Up and Down method was 0.54 μg/kg. The 7 day LD_{50} is the same, as no rabbits died between 48 hours and 7 days. The minimal lethal dose was 0.44 μg/kg, and 0.22 μg/kg was chosen as the toxic sub-lethal dose. These latter two doses were used during the following experiments: Blood pressure, ECG, heart rate, blood flow and blood flow distribution, norepinephrine content of aorta and plasma, monoamine oxidase activity, catechol-*O*-methyl transferase activity and cyclic AMP activity. At this time the supplier of rabbits changed. We found that even though the new rabbits were also male New Zealand White rabbits, their sensitivity to ricin was greater than that of the earlier rabbits. A new minimum lethal dose was determined to be 0.22 μg/kg and the new toxic sub-lethal dose was set at 0.11 μg/kg. These doses of ricin were used during the experiments on contractions and relaxations of arteries, norepinephrine uptake and efflux, and calcium uptake and efflux.

B. Effects of the Minimum Lethal and Toxic Sub-lethal Doses of Ricin on Rabbit Blood Pressure, Heart Rate, and ECG Patterns

1. Observations for Hours 12 through 48.

a. Blood Pressure

Blood pressure of rabbits given the toxic sub-lethal dose did not differ significantly from control rabbits during hours 12 through 48 following i.v. injection of ricin or sham injection. This was true of both the systolic pressure (Figure 1) and the diastolic pressure (Figure 2). Although differences were not significant, with the systolic pressure, examining each value from 22 hours and onward, at 20 of the time points the pressure was lower in the ricin-injected rabbits, while at only 6 of the time points were

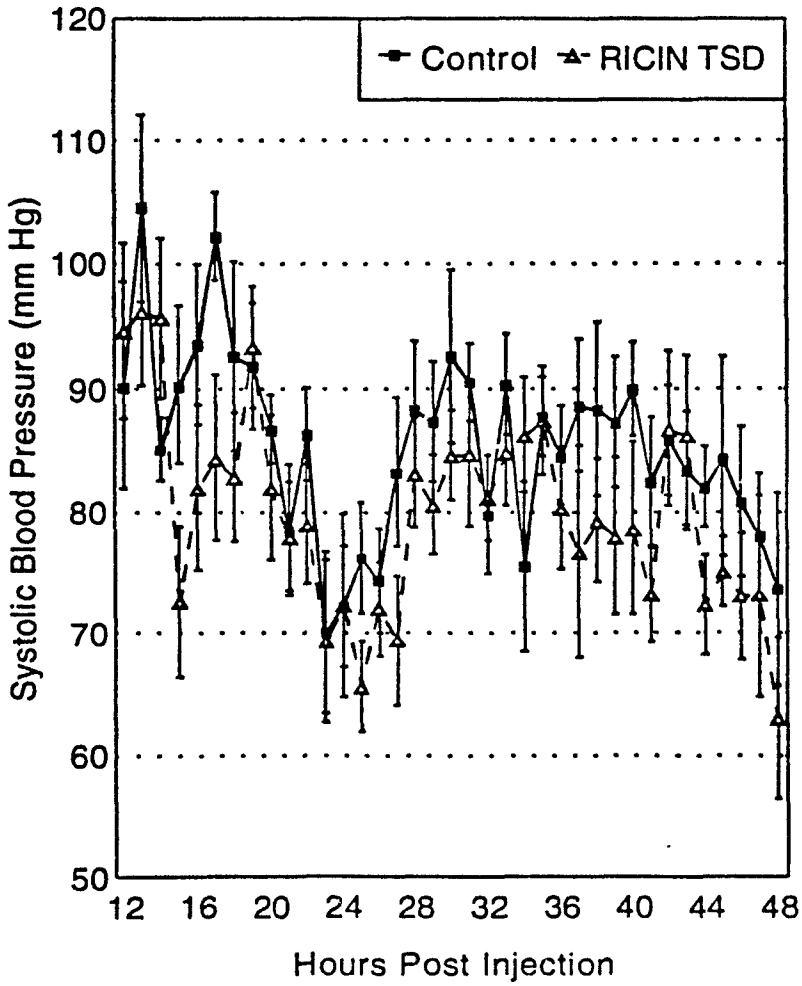

Figure 1. The effects of a toxic sub-lethal dose of ricin on the systolic blood pressure (mean ± S.E.M.) of 6 rabbits. Pressures were obtained each hour from 12 to 48 hours after i.v. ricin or sham-injection.

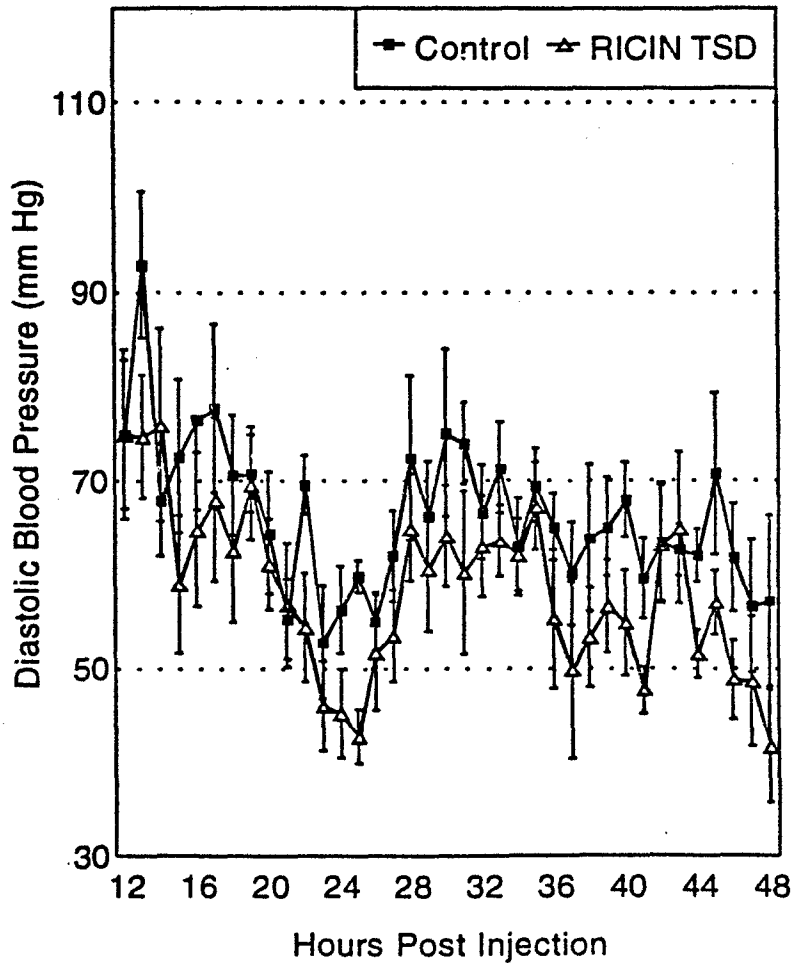

Figure 2. The effects of a toxic sub-lethal dose of ricin on the diastolic blood pressure (mean ± S.E.M.) of 6 rabbits. Pressures were obtained each hour from 12 to 48 hours after i.v. ricin or sham-injection.

control values lower. With the diastolic pressure the same tendency was observed; at 22 of the time points the pressure was lower in the ricin-injected rabbits, while at only 4 of the time points were blood pressures equal to or lower in control rabbits.

Following the i.v. injection of a minimum lethal dose of ricin, both the systolic (Figure 3) and diastolic (Figure 4) pressures fell significantly. Differences in pressures between control and ricin-treated rabbits became more marked with time, especially at around 24 hours post-ricin for the diastolic pressure and about 28 hours post-ricin for the systolic pressure.

b. ECG

There were no arrythymias detected during the observation periods following either the minimal lethal or toxic sub-lethal dose of ricin. (Data not shown).

c. Heart Rate

Both a minimum lethal dose and a toxic sub-lethal dose of ricin increased rabbit heart rate (Figures 5 and 6), although neither was signficantly different from control ($p > 0.05$). The heart rate at all 36 time points was higher than the control for the rabbits in the toxic sub-lethal dose group and 27 of the 36 measurements were higher than the control for the rabbits in the minimum lethal dose group. This increased heart rate could be a mechanism to compensate for the decreasing blood pressure.

2. Observations for Hours 1 Through 12, (on Another Group of Rabbits)

a. Blood Pressure

There were no consistent changes in blood pressures throughout the first 12 hours following the i.v. injection of either the minimal lethal or toxic sub-lethal dose of ricin (Figures 7-10).

C. Pathological Changes Following Ricin Injection into Rabbits

Three rabbits showed signs of excessive salivation. Two of the rabbits died at 24 hours, while the other one died at approximately 40 hours post-

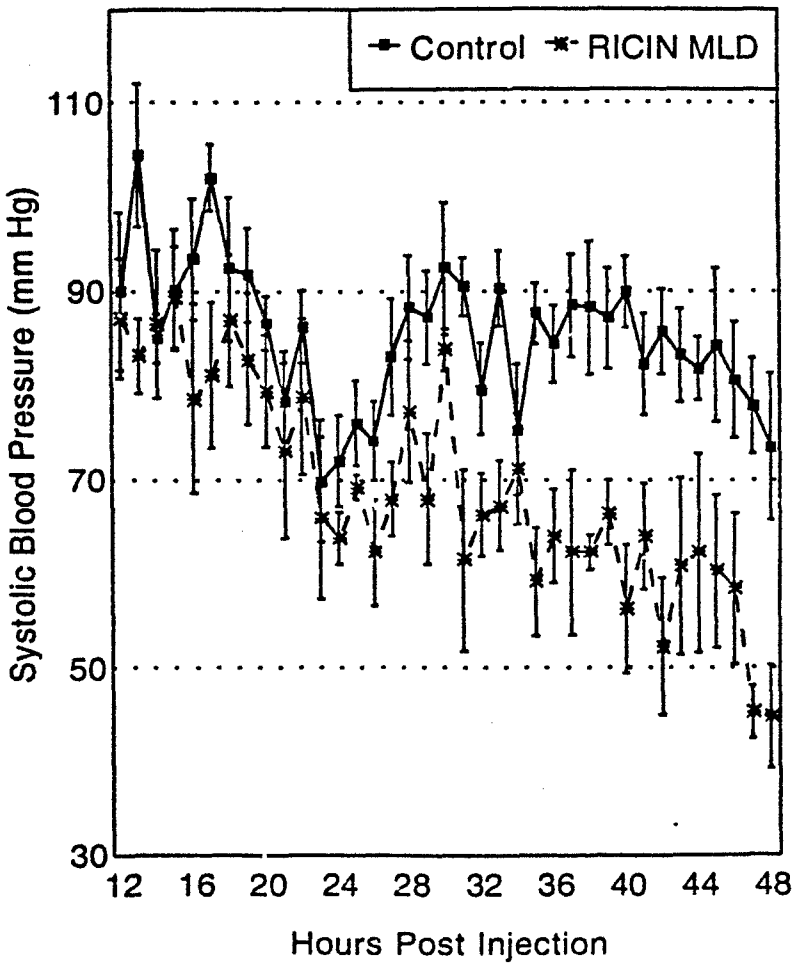

Figure 3. The effects of the minimum lethal dose of ricin on the systolic blood pressure (mean ± S.E.M.) of 6 rabbits. Pressures were obtained each hour from 12 to 48 hours after i.v. ricin or sham-injection.

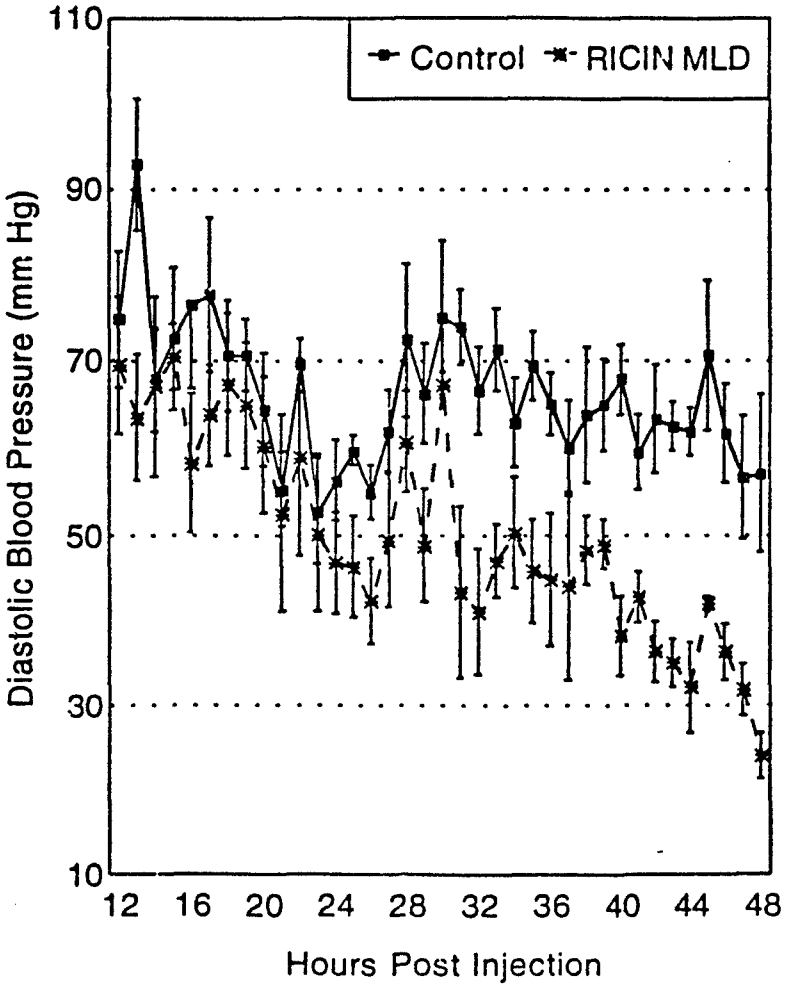

Figure 4. The effects of the minimum lethal dose of ricin on the diastolic blood pressure (mean ± S.E.M.) of 6 rabbits. Pressures were obtained each hour from 12 to 48 hours after i.v. ricin or sham-injection.

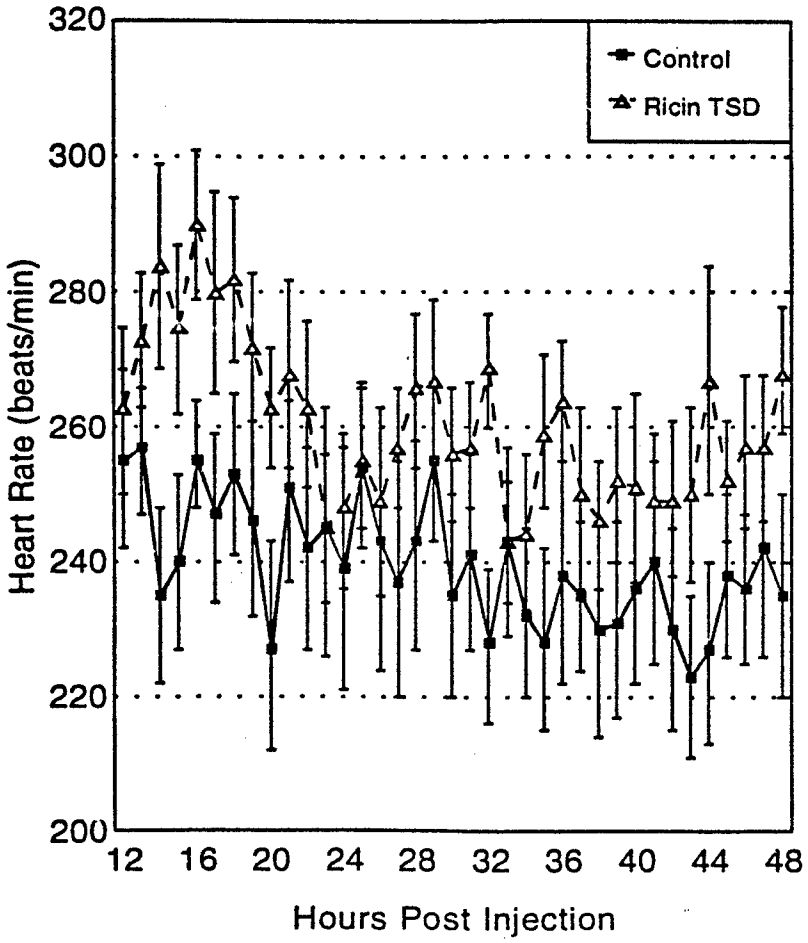

Figure 5. The effects of a toxic sub-lethal dose of ricin on the heart rate (mean ± S.E.M.) of 7 rabbits. Heart rates were obtained each hour from 12 to 48 hours after i.v. ricin or sham-injection.

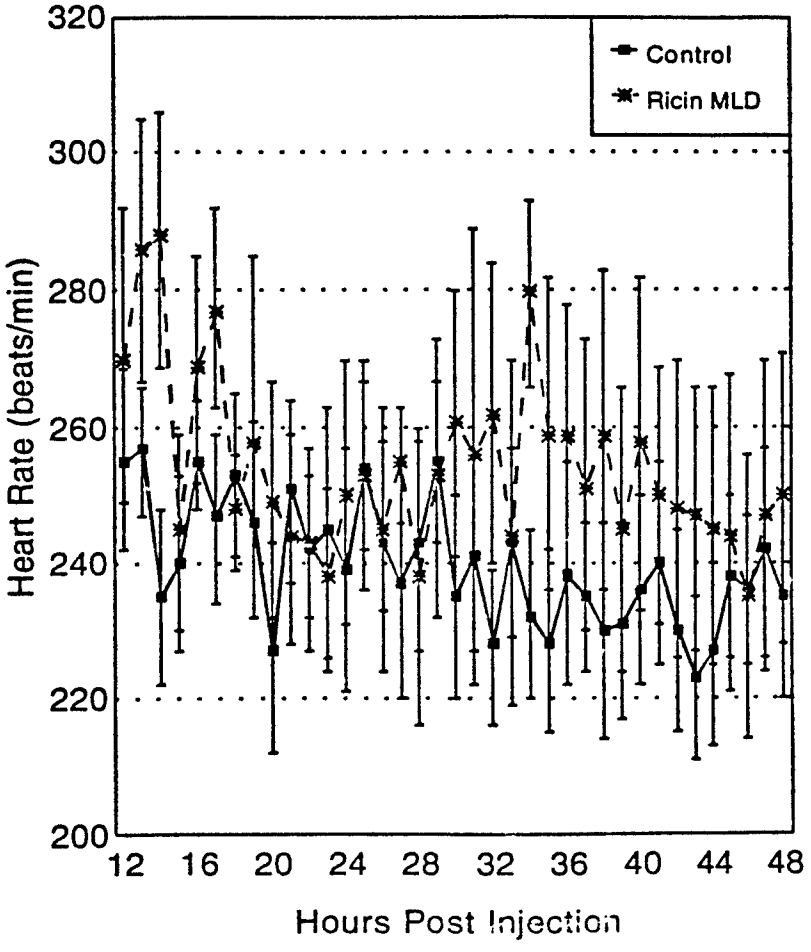

Figure 6. The effects of a minimum lethal dose of ricin on the heart rate (mean ± S.E.M.) of 7 rabbits. Heart rates were obtained each hour from 12 to 48 hours after i.v. ricin or sham-injection.

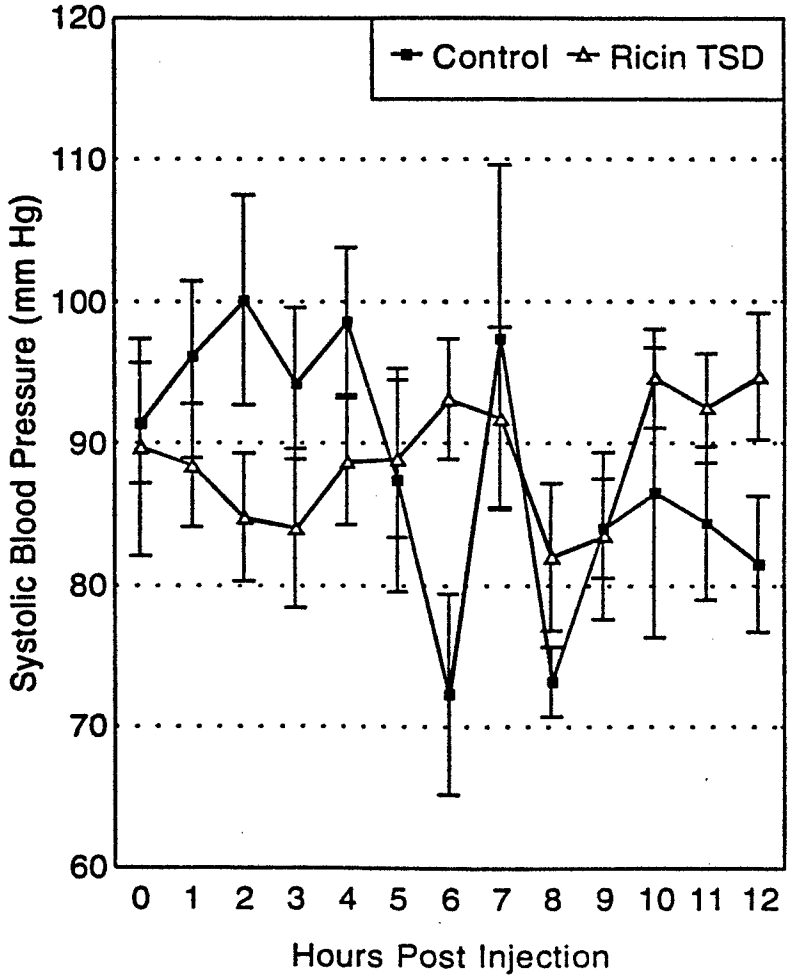

Figure 7. The effects of a toxic sub-lethal dose of ricin on the systolic blood pressure (mean ± S.E.M.) of 6 rabbits. Pressures were obtained each hour for 12 hours after i.v. ricin or sham-injection.

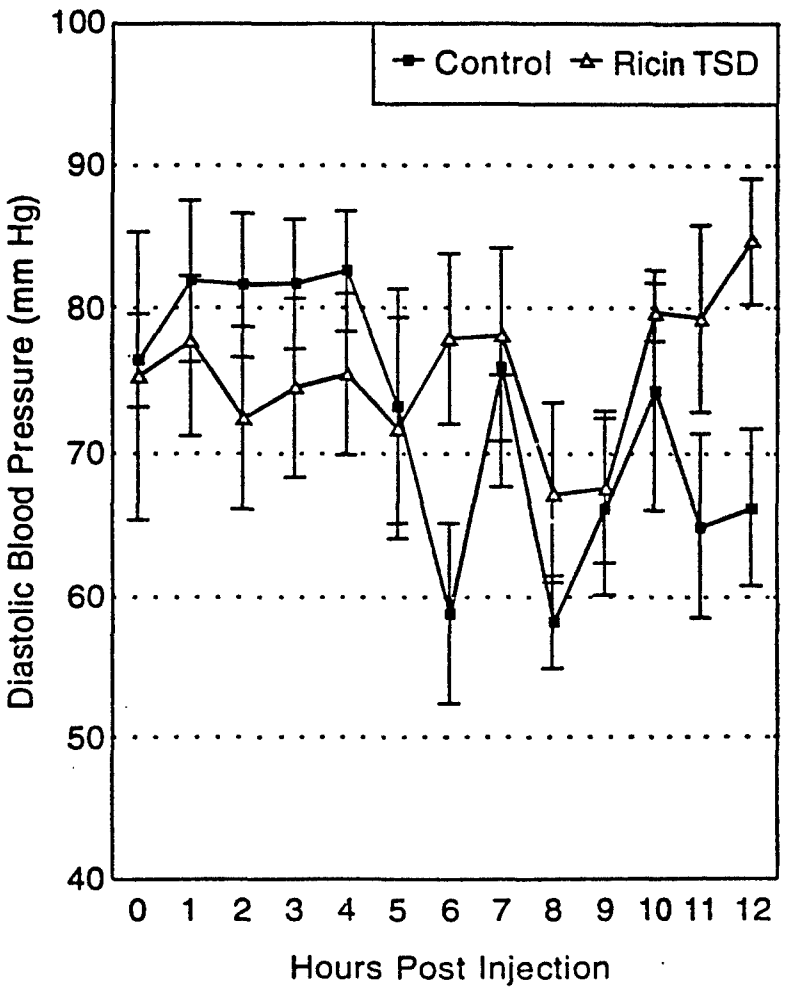

Figure 8. The effects of a toxic sub-lethal dose of ricin on the diastolic blood pressure (mean ± S.E.M.) of 6 rabbits. Pressures were obtained each hour for 12 hours after i.v. ricin or sham-injection.

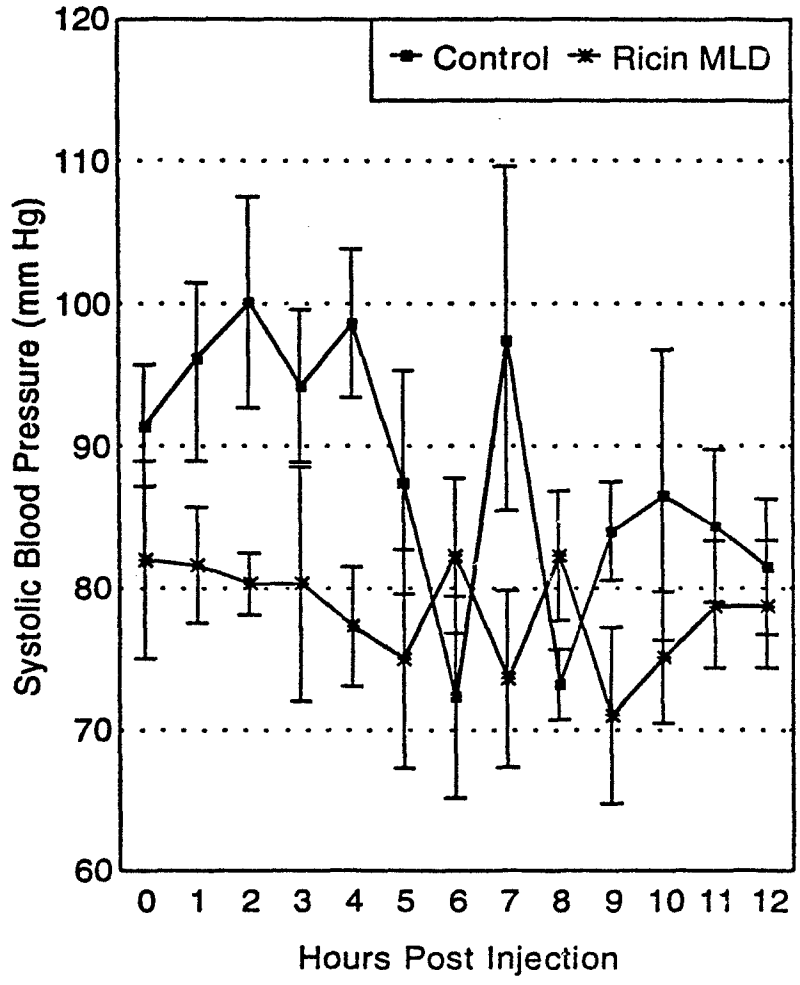

Figure 9. The effects of a minimum lethal dose of ricin on the systolic blood pressure (mean ± S.E.M.) of 6 rabbits. Pressures were obtained each hour for 12 hours after i.v. ricin or sham-injection.

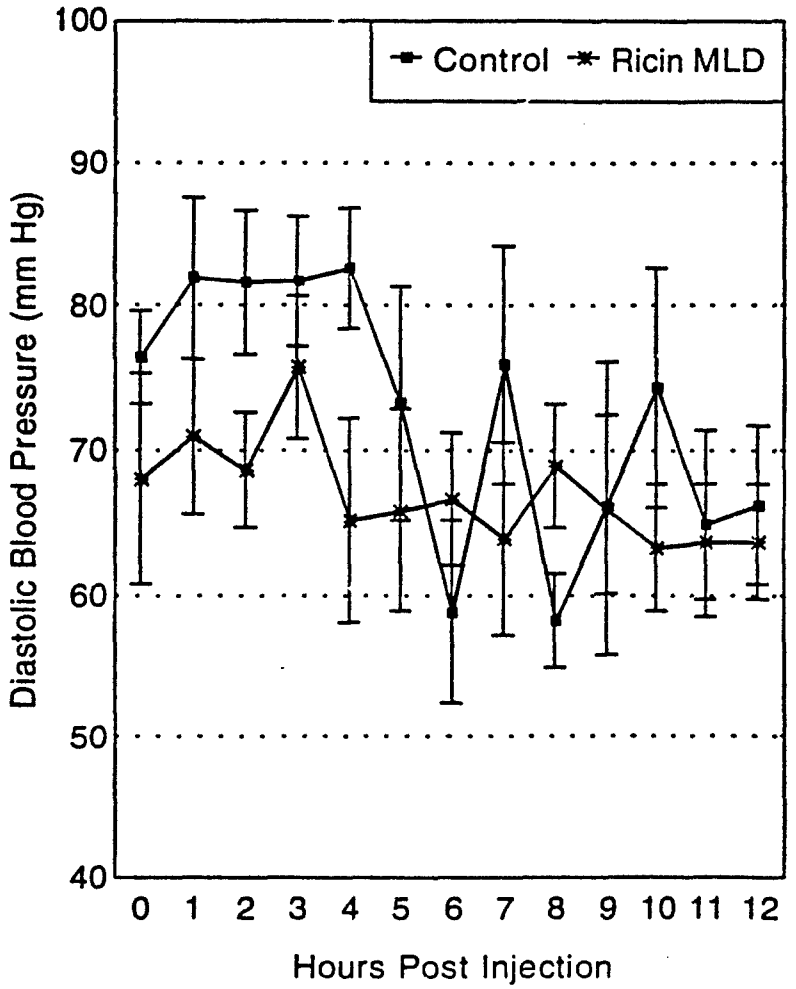

Figure 10. The effects of a minimum lethal dose of ricin on the diastolic blood pressure (mean ± S.E.M.) of 6 rabbits. Pressures were obtained each hour for 12 hours after i.v. ricin or sham-injection.

ricin. The one that lived 40 hours no longer displayed excessive salivation at the time of death. At least one rabbit convulsed at the time of death.

Pathological changes were observed in many tissues. Following the 0.57 μg/kg ricin injection (higher than the minimum lethal dose), two rabbits died between 22 and 24 hours, one at 36 hours, and two between 44 and 48 hours. The three rabbits that survived the longest times following ricin administration developed severe coagulative necrosis of the centrilobular zones of the liver (Figures 11-15) and severe mucosal erosion of the stomach (Figures 16, 17) characterized by hemorrhage and necrosis. Much milder effects on the liver (Figure 18) and stomach were observed in rabbits surviving only 24 hours. Hemorrhage involving the myocardium, renal pelvis, and urinary bladder mucosa was also present only in rabbits surviving 48 hours. Rabbits surviving only 24 (Figure 19) or 36 (Figure 20) hours had severe pulmonary congestion, edema and hemorrhage. Severe hemorrhage was observed in the heart of a rabbit that survived almost 48 hours (Figure 21). Thymic (Figure 22) and lymph node congestion were also observed in the study.

The hepatic damage, severe coagulative necrosis in the centrilobular and paraventral regions with sparing of the periportal area, is typical of damage due to low blood flow to the liver such as in congestive heart failure. The severe hemorrhage of the heart muscle would suggest a decrease in contractility, and severe edema in the lungs would also suggest a backing up of fluid due to decreased cardiac contractility. Microsphere studies showed an increase in cardiac output and blood flow to the liver. However, these studies were only done at 12 and 18 hours post-ricin, and neither heart nor liver damage appeared in the animals that died at 24 hours post-ricin. Therefore, the liver damage may be due to a decreased blood flow secondary to a failing heart.

Although these findings are interesting, it must be borne in mind that these data are from only five rabbits.

D. Alterations in Laboratory Values Following Administration of Ricin to Male Rabbits

Laboratory values were determined for control male rabbits and those given a toxic sub-lethal dose, a minimum lethal dose, or 0.57 μg/kg (higher

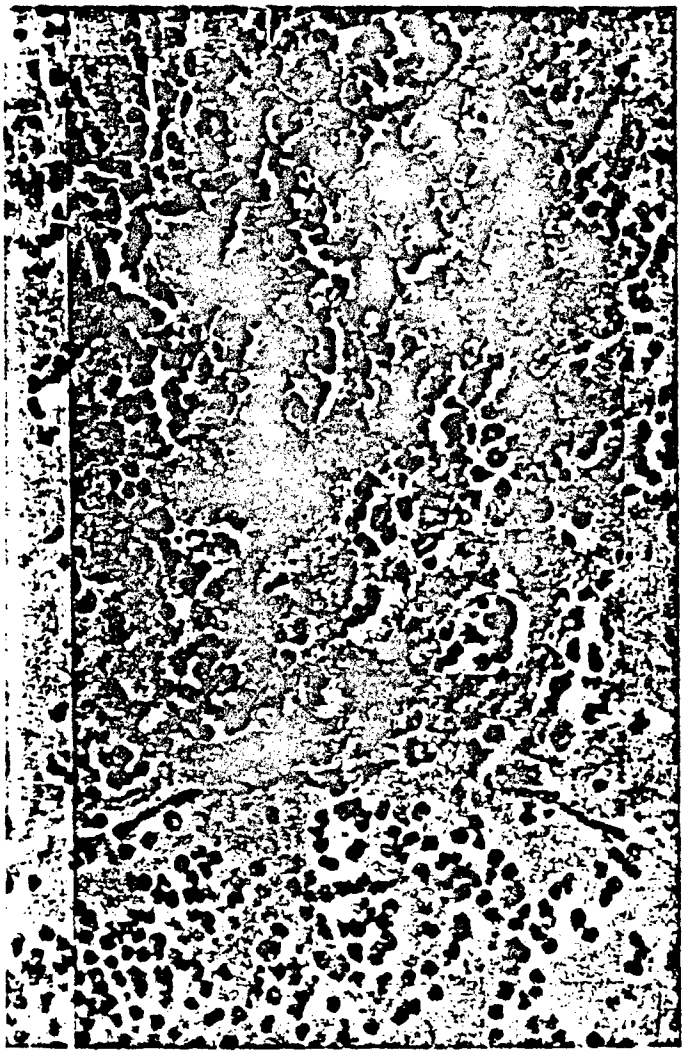

Figure 11. Liver from a rabbit that died 36 hours after ricin injection. Severe coagulative necrosis with acute inflammation was present in zone 3.

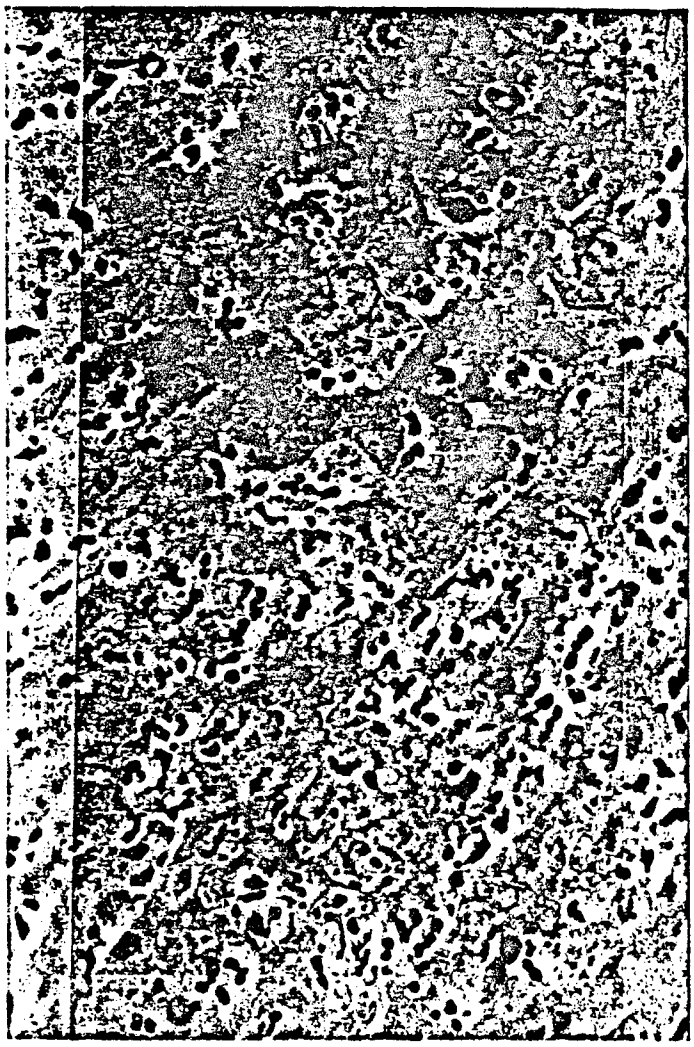

Figure 12. Same rabbit as in Figure 11. Severe coagulative necrosis with acute inflammation was also present in zone 2 (paracentral).

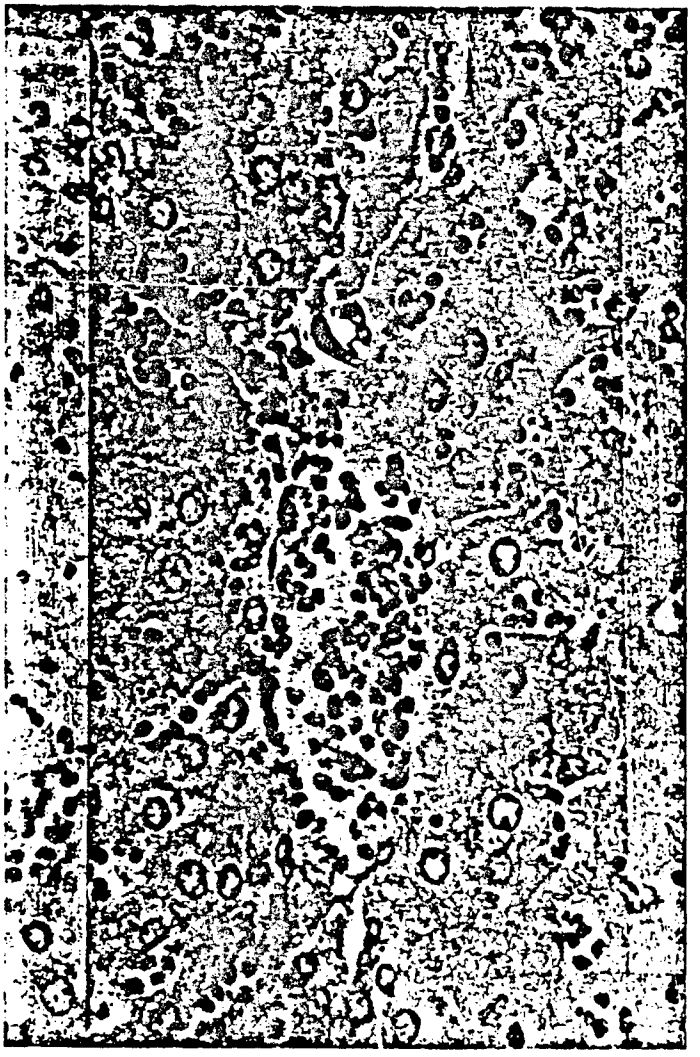

Figure 13. Liver from a rabbit that died 48 hours after ricin injection. Coagulative necrosis, acute inflammation and congestion involving zone 3 was observed.

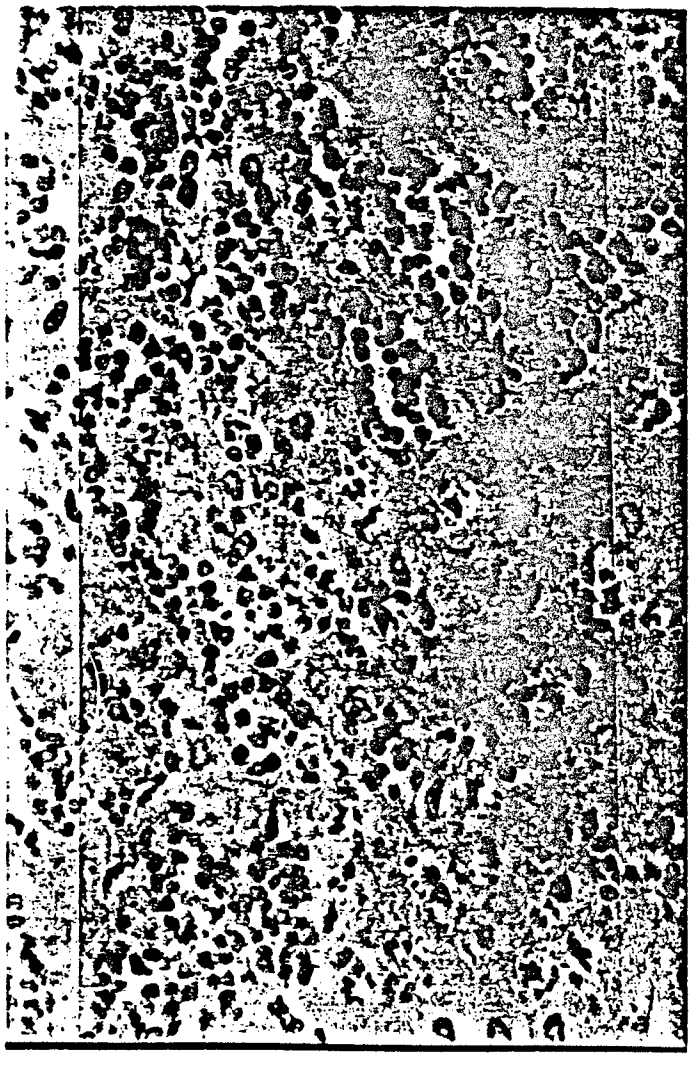

Figure 14. Liver from a rabbit that died 48 hours after ricin injection. (The same rabbit as in Figure 13). Coagulative necrosis, acute inflammation and congestion involving zone 2 were also observed.

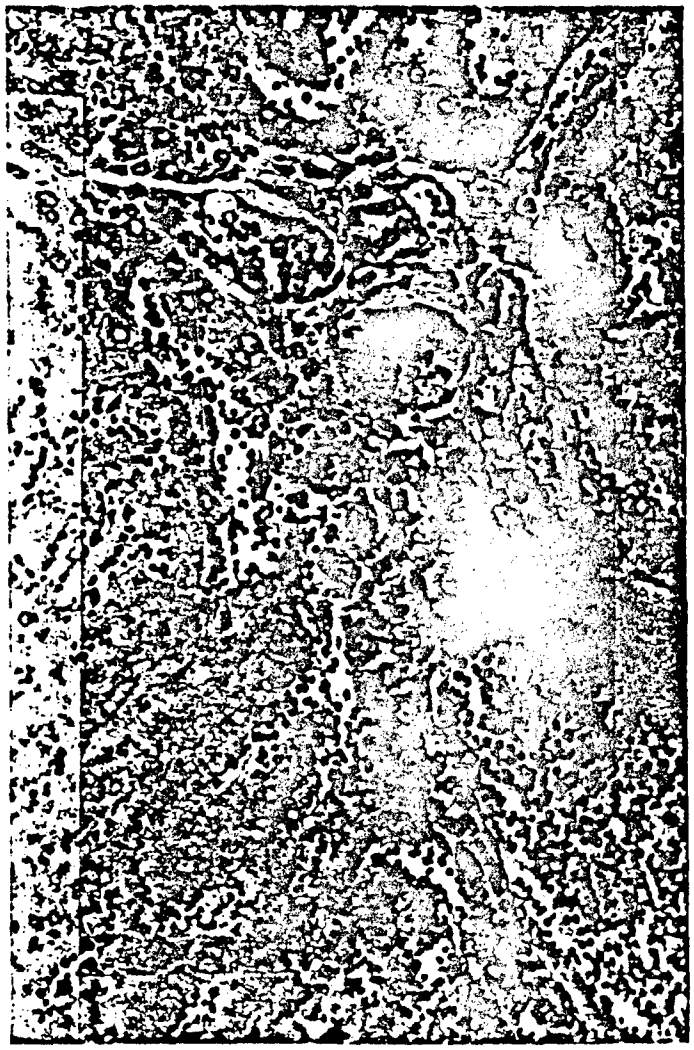

Figure 15. Liver from a rabbit that died 48 hours after ricin injection. Zone 1 (periportal) is essentially spared. Zone 2 reveals severe coagulative necrosis.

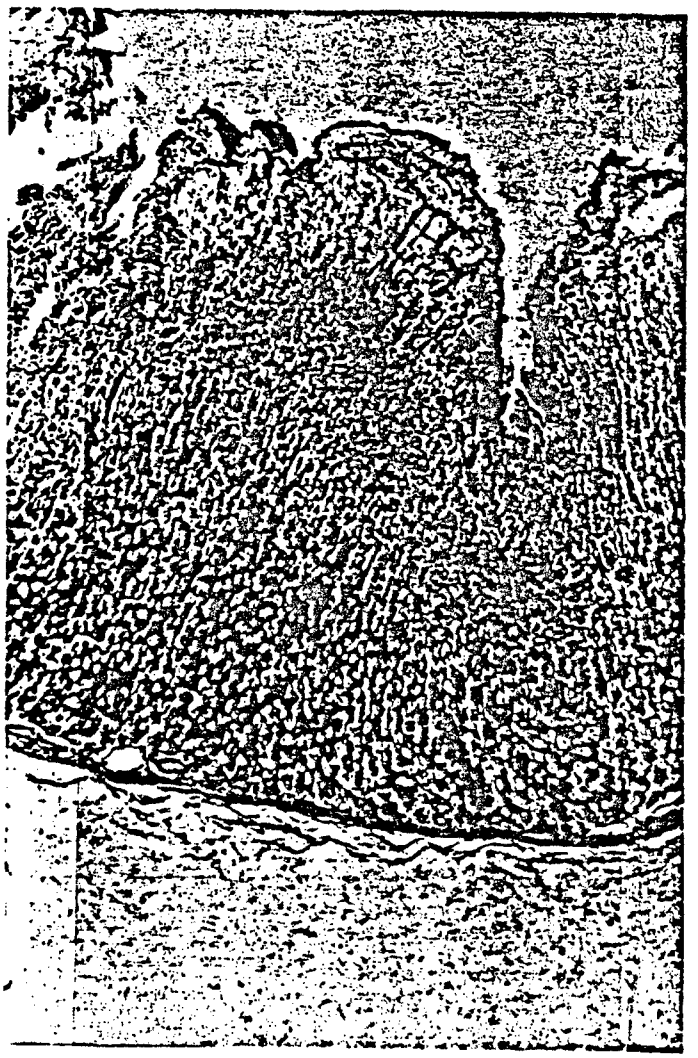

Figure 16. Stomach from a rabbit that died 48 hours after ricin injection. Severe mucosal erosion was characterized by hemorrhage and necrosis.

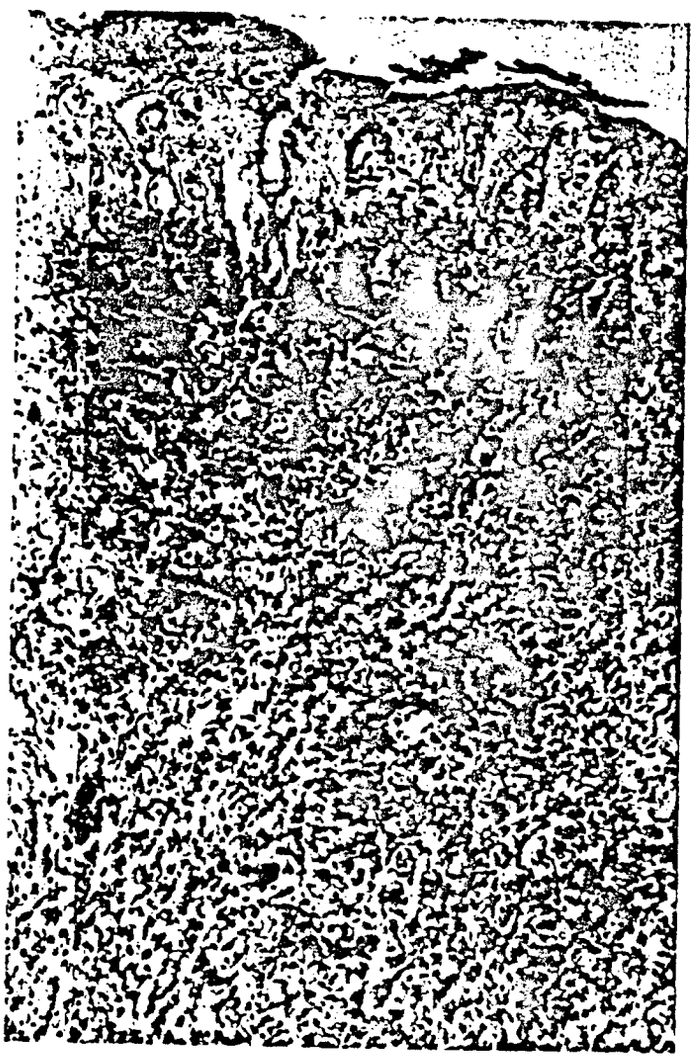

Figure 17. Stomach from the rabbit in Figure 16, but a different magnification.

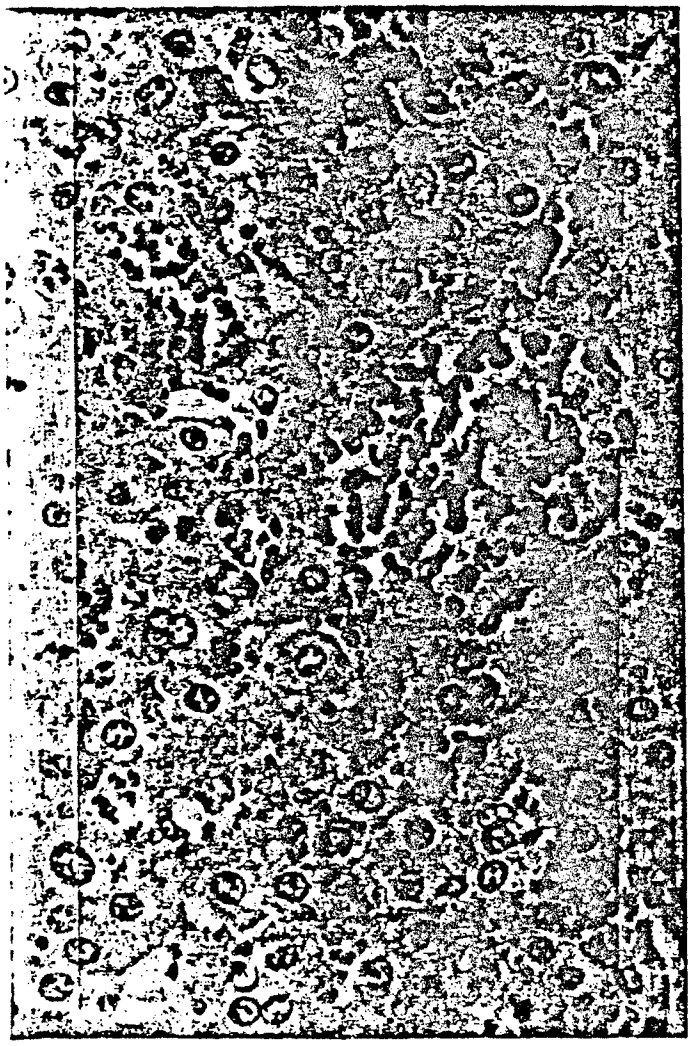

Figure 18. Liver from a rabbit that died 24 hours after ricin injection. Central vein and sinusoidal congestion with early piecemeal hepatocyte necrosis in the centrilobular (Zone 3) region was observed.

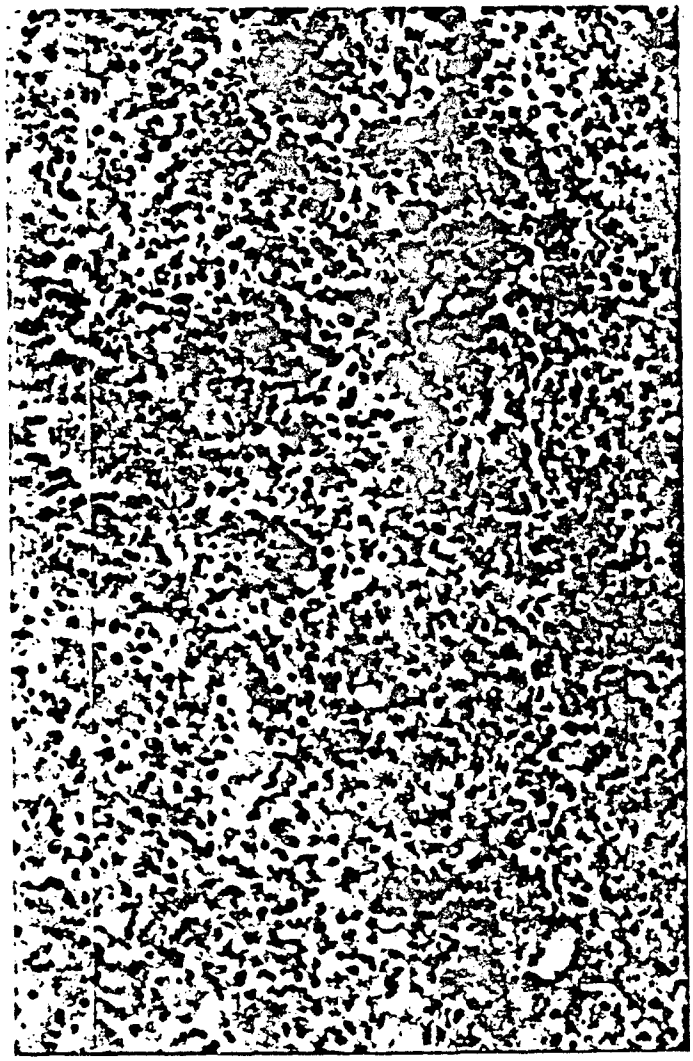

Figure 19. Lung from a rabbit that died 24 hours after ricin injection. Massive congestion, edema and hemorrhage were observed. Alveolar spaces were filled with proteinaceous fluid, fibrin and red blood cells. Compare with the rabbit that lived for 36 hours.

Figure 20. Lung from a rabbit that died 36 hours after ricin injection. Moderate to severe congestion without hemorrhage was present.

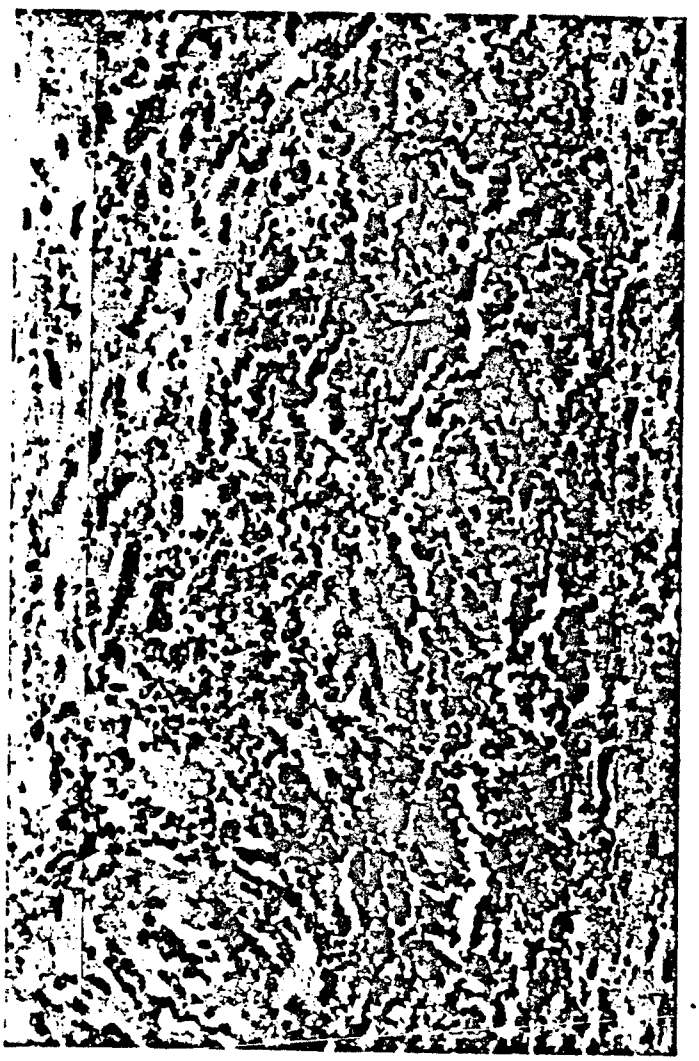

Figure 21. Heart from a rabbit that died 48 hours after ricin injection. Severe hemorrhage was present in the myocardium.

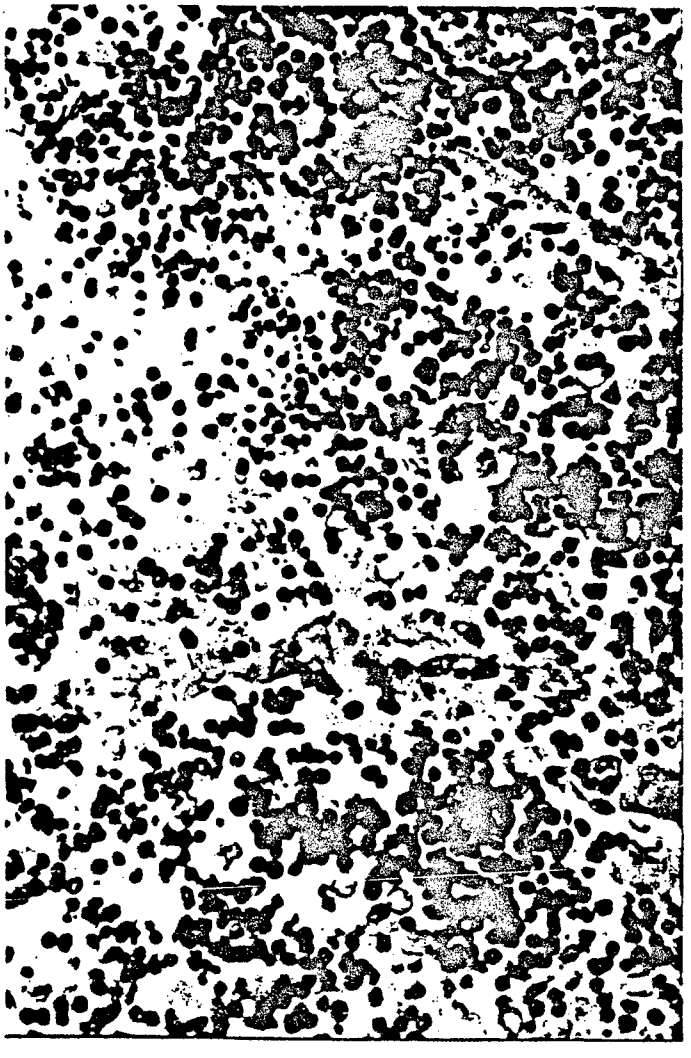

Figure 22. Thymus from a rabbit that died 36 hours after ricin injection. Moderate congestion and hemorrhage with mild necrosis were present.

than a minimum lethal dose) of ricin. (Tables 1-4)

Of the 19 laboratory values determined, only plasma calcium concentrations were consistently reduced.

There were four values that were consistently elevated: LDH, CPK, SGPT, and cholesterol.

E. Lethality And Changes in Laboratory Values Following Administration of Ricin to Older Female Rabbits

Laboratory values were determined for older female rabbits given a minimum lethal dose of ricin. As in the male rabbits, serum LDH, CPK, SGPT, and cholesterol were elevated, while calcium concentrations were reduced (Table 5).

F. Effects of Ricin Administration on Blood Flow and Blood Flow Distribution Using Radio-labeled Microspheres

Ricin increased cardiac output at both concentrations employed (Figure 23). The toxic sub-lethal dose of ricin increased blood flow by 28% at 12 hours after injection and by 29% at 18 hours. The minimal lethal dose increased blood flow by 33% at 12 hours but then blood flow decreased slightly to 27% above the control value at 18 hours post-ricin.

Ricin increased blood flow to most tissues (Tables 6 and 7, Figure 24-37). The brain is somewhat of an exception to that. Twelve hours after injection of a toxic sub-lethal dose of ricin, blood flow to all areas of the brain had markedly increased, but six hours later, blood flow to the various areas had substantially returned to normal (Figure 26). The minimal lethal dose, however, decreased the blood flow to the brain at 12 hours, and markedly decreased its blood flow at 18 hours, to 36% of the initial blood flow.

Another exception is the lungs. Because the microspheres were injected into the left ventricle, the lungs would only receive microspheres which were not trapped by lodging in capillaries. These presumably would, for the most part, arrive there by either passing through arterio-venous

Table 1. Laboratory Values[a] From Rabbits Receiving a Minimum Lethal Dose of Ricin

	TIME (IN HOURS) AFTER INJECTION				
	0	12	24	36	48
	159 ± 67 n = 4	176 ± 27 n = 2	271 ± 162 n = 3	513 ± 570 n = 3	211 ± 118 n = 2
CPK	587 ± 451 n = 4	1791 ± 140 n = 2	1345 ± 458 n = 3	1724 ± 1150 n = 3	1014 ± 323 n = 2
BUN	23 ± 5 n = 4	14 ± 1 n = 2	33 ± 26 n = 3	36 ± 19 n = 3	24 ± 2 n = 2
CALCIUM	16.0 ± 1.8 n = 4	14.0 ± 2.1 n = 2	11.5 ± 2.8 n = 3	11.8 ± 1.3 n = 3	12.9 ± 2.3 n = 2
GLUCOSE	145 ± 4 n = 4	193 ± 22 n = 2	179 ± 31 n = 3	205 ± 23 n = 3	158 ± 6 n = 2
PHOSPH-ORUS	5.9 ± 0.9 n = 4	6.7 ± 1.2 n = 2	6.1 ± 1.2 n = 3	8.6 ± 1.5 n = 3	6.3 ± 0.2 n = 2
TOTAL PROTEIN	6.3 ± 0.5 n = 3	6.0 ± 0 n = 2	6.2 ± 0.6 n = 3	6.0 ± 0.5 n = 3	5.1 ± 0.3 n = 2
ALBUMIN	4.2 ± 0.1 n = 3	4.5 ± 0.5 n = 2	3.9 ± 0.3 n = 3	4.1 ± 0.5 n = 3	3.3 ± 0.2 n = 2
GLOBU-LIN	2.7 ± 0.5 n = 3	1.5 ± 0.5 n = 2	2.3 ± 0.4 n = 3	1.9 ± 1 n = 3	1.8 ± 0.1 n = 2
A/G RATIO	2.1 ± 0.5 n = 3	3.5 ± 1.5 n = 2	1.8 ± 0.3 n = 3	2.1 ± 0.2 n = 3	1.8 ± 0 n = 2
CHOLES-TEROL	41 ± 17 n = 3	49 ± 6 n = 2	65 ± 14 n = 3	96 ± 41 n = 3	81 ± 21 n = 2
TOTAL BILIRU	0.85 ± 0.23 n = 4	0.4 ± 0.1 n = 2	1.1 ± 0.5 n = 3	0.7 ± 0.2 n = 3	0.85 ± 0.25 n = 2
ALK PHOS	155 ± 74 n = 4	198 ± 28 n = 2	121 ± 48 n = 3	145 ± 28 n = 3	93 ± 1 n = 2

Table 1 (cont'd). Laboratory Values[a] From Rabbits Receiving a Minimum Lethal Dose of Ricin.

	TIME (IN HOURS) AFTER INJECTION				
	0	12	24	36	48
SGPT (ALT)	43 ± 8 n = 4	30 ± 5 n = 2	50 ± 11 n = 3	66 ± 25 n = 3	50 ± 4 n = 2
GGT	8.3 ± 2.9 n = 4	15.5 ± 14.5 n = 2	27.3 ± 18.8 n = 3	12.7 ± 6.3 n = 3	8 ± 4 n = 2
CREATI-NINE	1.2 ± 0.36 n = 4	0.75 ± 0.25 n = 2	2.0 ± 1.20 n = 3	0.8 ± 0.17 n = 3	8.5 ± 0.15 n = 2
AMYLASE	313 ± 38 n = 4	186 ± 15 n = 2	283 ± 68 n = 3	300 ± 17 n = 3	282 ± 61 n = 2
SODIUM	146 ± 3 n = 4	166 ± 11 n = 2	145 ± 1 n = 2	167 ± 13 n = 3	139 ± 3 n = 2
POTASS-IUM	5.15 ± 0.2 n = 4	5.05 ± 0.45 n = 2	4.2 ± 0.9 n = 2	4.7 ± 0.6 n = 3	4.45 ± 0.65 n = 2

a = Units (mean ± S.E.M.) for the values are: Lactate dehydrogenase, IU/L; creatine phosphokinase, IU/L; blood urea nitrogen, mg/DL; calcium, mg/DL; glucose, mg/DL; phosphorus, mg/DL; total protein, g/DL; albumin, g/DL; globulin, g/DL; albumin/globulin ratio; cholesterol, mg/DL; total bilirubin, mg/DL; alkaline phosphatase, IU/L; serum glutamic pyruvic transaminase, IU/L; gamma glutamyl transpeptidase, IU/L; creatinine, mg/DL; amylase, IU/L; sodium, mEq/L; potassium, mEq/L.

Table 2. Laboratory Values[a] From Rabbits Receiving a Toxic Sub-lethal Dose of Ricin.

	TIME (IN HOURS) AFTER INJECTION				
	0	12	24	36	48
LDH	109 ± 50 n = 3	115 ± 59 n = 2	234 ± 213 n = 2	407 ± 524 n = 3	343 ± 152 n = 3
CPK	828 ± 834 n = 3	1471 ± 119 n = 2	1438 ± 21 n = 2	1456 ± 594 n = 2	1356 ± 613 n = 3
BUN	15.7 ± 5.5 n = 3	12.0 ± 2.8 n = 2	17.0 ± 1.4 n = 2	21.0 ± 9 n = 3	19.3 ± 9.3 n = 3
CALCIUM	15.5 ± 0.4 n = 3	14.2 ± 0.07 n = 2	12.4 ± 2.7 n = 2	14.5 ± 1.6 n = 3	12.2 ± 3.2 n = 3
GLUCOSE	177 ± 11 n = 3	156 ± 8 n = 2	156 ± 23 n = 2	201 ± 20 n = 3	165 ± 2 n = 3
PHOS-PHORUS	7.8 ± 0.6 n = 3	7.4 ± 0.3 n = 2	5.8 ± 0.6 n = 2	6.2 ± 0.2 n = 3	4.7 ± 0.3 n = 3
TOTAL PROTEIN	5.7 ± 0.3 n = 3	5.6 ± 0.45 n = 2	5.6 ± 0.0 n = 2	6.5 ± 0.7 n = 3	5.8 ± 0.3 n = 3
ALBUMIN	3.9 ± 0.03 n = 3	4.0 ± 0.15 n = 2	3.7 ± 0.1 n = 2	4.6 ± 0.7 n = 3	3.8 ± 0.4 n = 3
GLOBU-LIN	1.7 ± 0.3 n = 3	1.6 ± 0.3 n = 2	1.9 ± 0.1 n = 2	1.9 ± 0.03 n = 3	2.0 ± 0.03 n = 3
A/G RATIO	2.4 ± 0.4 n = 3	2.6 ± 0.35 n = 2	1.95 ± 0.15 n = 2	2.5 ± 0.4 n = 3	1.9 ± 0.2 n = 3
CHOLES-TEROL	33 ± 7 n = 3	44 ± 15 n = 2	37 ± .05 n = 2	49 ± 12 n = 3	60 ± 13 n = 2
TOTAL BILIRU	0.5 ± 0.14 n = 3	0.35 ± 0.4 n = 2	0.7 ± 0.5 n = 2	0.67 ± 0.6 n = 3	0.76 ± 0.18 n = 3
ALK PHOS	164 ± 28 n = 3	162 ± 23 n = 2	134 ± 22 n = 2	145 ± 18 n = 3	89 ± 8 n = 3

Table 2 (cont'd). Laboratory Values[a] From Rabbits Receiving a Toxic Sub-lethal Dose of Ricin.

	TIME (IN HOURS) AFTER INJECTION				
	0	12	24	36	48
SGPT (ALT)	38 ± 7 n = 3	35 ± 8 n = 2	48 ± .05 n = 2	52 ± 4 n = 3	46 ± 5 n = 3
GGT	5.3 ± 2.6 n = 3	5. ± 4.0 n = 2	11.5 ± 3.5 n = 2	5.3 ± 1.9 n = 3	7.0 ± 2.0 n = 2
CREATI-NINE	1.07 ± 0.30 n = 3	0.75 ± 0.15 n = 2	0.9 ± 0 n = 2	0.7 ± 0.17 n = 3	0.87 ± 0.12 n = 3
AMYLASE	225 ± 30 n = 3	209 ± 8 n = 2	231 ± 37 n = 2	261 ± 29 n = 3	269 ± 52 n = 3
SODIUM	143 ± 2 n = 3	149 ± 0.5 n = 2	140 ± 5 n = 2	144 ± 10 n = 2	120 ± 44 n = 3
POTASS-IUM	4.5 ± 0.17 n = 3	4.4 ± 0.1 n = 2	4.85 ± 0.75 n = 2	4.1 ± 0.4 n = 2	6.7 ± 1.7 n = 3

[a] = Units (mean ± S.E.M.) for the values are: Lactate dehydrogenase, IU/L; creatine phosphokinase, IU/L; blood urea nitrogen, mg/DL; calcium, mg/DL; glucose, mg/DL; phosphorus, mg/DL; total protein, g/DL; albumin, g/DL; globulin, g/DL; albumin/globulin ratio; cholesterol, mg/DL; total bilirubin, mg/DL; alkaline phosphatase, IU/L; serum glutamic pyruvic transaminase, IU/L; gamma glutamyl transpeptidase, IU/L; creatinine, mg/DL; amylase, IU/L; sodium, mEq/L; potassium, mEq/L.

Table 3. Laboratory Values[a] From Rabbits Receiving a Sham Injection.

	TIME (IN HOURS) AFTER INJECTION				
	0	12	24	36	48
LDH	239 ± 181 n = 4	237 n = 1	164 ± 81 n = 2	321 ± 127 n = 2	156 n = 1
CPK	1227 ± 1352 n = 4	2049 n = 1	1117 ± 578 n = 2	2179 ± 1093 n = 2	2059 n = 1
BUN	18 ± 3.5 n = 4	11 n = 1	15.5 ± 2.1 n = 2	21 ± 7.1 n = 2	13 n = 1
CALCIUM	15 ± 2.4 n = 4	14.8 n = 1	14.5 ± 1.3 n = 2	13.8 ± 0.35 n = 2	13.8 n = 1
GLUCOSE	170 ± 11 n = 4	1821 n = 1	161 ± 18 n = 2	167 ± 25 n = 2	158 n = 1
PHOS-PHORUS	9.2 ± 2.5 n = 4	6.1 n = 1	6.2 ± 0.3 n = 2	4.7 ± 1.1 n = 2	6.8 n = 1
TOTAL PROTEIN	5.9 ± 0.1 n = 4	5.1 n = 1	6.0 ± 0.2 n = 2	6.3 ± 0.2 n = 2	6.2 n = 1
ALBUMIN	4.4 ± 0.2 n = 4	3.9 n = 1	4.2 ± 0.05 n = 2	4.3 ± 0.05 n = 2	4.2 n = 1
GLOBU-LIN	1.5 ± 0.2 n = 4	1.2 n = 1	1.9 ± 0.15 n = 2	2.0 ± 0.1 n = 2	2.0 n = 1
A/G RATIO	3.0 ± 0.6 n = 4	3.3 n = 1	2.3 ± 0.15 n = 2	2.2 ± 0.05 n = 2	2.1 n = 1
CHOLES-TEROL	36 ± 15 n = 4	64 n = 1	38 ± 15 n = 2	90 ± 21 n = 2	52 n = 1
TOTAL BILIRU	1.0 ± 0.5 n = 4	0.8 n = 1	0.25 ± 0.25 n = 2	1.8 ± 1.1 n = 2	0.0 n = 1
ALK PHOS	160 ± 27 n = 4	193 n = 1	145 ± 53 n = 2	129 ± 60 n = 2	91 n = 1

Table 3 (cont'd). Laboratory Values[a] From Rabbits Receiving a Sham-Injection.

	TIME (IN HOURS) AFTER INJECTION				
	0	12	24	36	48
SGPT (ALT)	45 ± 11 n = 4	27 n = 1	44 ± 20 n = 2	31 ± 6 n = 2	64 n = 1
GGT	20.5 ± 13.3 n = 4	5.0 n = 1	4.0 ± 2 n = 2	2.0 ± 1.0 n = 2	2.0 n = 1
CREATI-NINE	0.9 ± 0.17 n = 4	0.7 n = 1	0.8 ± 0.1 n = 2	0.9 ± 0.0 n = 2	0.5 n = 1
AMYLASE	261 ± 11 n = 4	224 n = 1	274 ± 52 n = 2	269 ± 8 n = 2	305 n = 1
SODIUM	143 ± 3 n = 4	148 n = 1	142 ± 1 n = 2	157 ± 17 n = 2	139 n = 1
POTASS-IUM	5.2 ± 0.5 n = 4	5.1 n = 1	4.6 ± 0.65 n = 2	5.4 ± 0.65 n = 2	4.1 n = 1

[a] = Units (mean S.E.M.) for the values are: Lactate dehydrogenase, IU/L; creatine phosphokinase, IU/L; blood urea nitrogen, mg/DL; calcium, mg/DL; glucose, mg/DL; phosphorus, mg/DL; total protein, g/DL; albumin, g/DL; globulin, g/DL; albumin/globulin ratio; cholesterol, mg/DL; total bilirubin, mg/DL; alkaline phosphatase, IU/L; serum glutamic pyruvic transaminase, IU/L; gamma glutamyl transpeptidase, IU/L; creatinine, mg/DL; amylase, IU/L; sodium, mEq/L; potassium, mEq/L.

Table 4. Laboratory Values[a] From Rabbits Receiving 0.57 μg/kg (higher than the minimum lethal dose) of Ricin.

	TIME (IN HOURS) AFTER INJECTION	
	0	12
LDH	995 ± 332 n = 3	388 ± 181 n = 3
CPK	1878 ± 297 n = 3	2419 ±1392 n = 3
BUN	16.7 ± 3.3 n = 3	16 ± 3.6 n = 3
CALCIUM	15.6 ± 0.9 n = 3	10.2 ± 1.1 n = 3
GLUCOSE	211 ± 25 n = 3	159 ± 2 n = 3
PHOS-PHORUS	5.4 ± 2.0 n = 3	7.3 ± 0.9 n = 3
TOTAL PROTEIN	6.7 ± 0.7 n = 3	5.6 ± 0.12 n = 3
ALBUMIN	4.6 ± 0.5 5 = 3	3.8 ± 0.06 n = 3
GLOBU-LIN	2.0 ± 0.2 n = 3	1.8 ± 0.09 n = 3
A/G RATIO	2.2 ± 0.2 n = 3	2.2 ± 0.1 n = 3
CHOLES-TEROL	4.7 ± 8.5 n = 3	70 ± 6.9 n = 3
TOTAL BILIRU	5.9 ± 3.5 n = 3	1.7 ± 9 n = 3
ALK PHOS	102 ± 29.0 n = 3	110 ± 10.4 n = 3

Table 4 (cont'd). Laboratory Values[a] From Rabbits Receiving 0.57 μg/kg (higher than a minimum lethal dose) of Ricin.

	TIME (IN HOURS) AFTER INJECTION 0	12
SGPT (ALT)	31 ± 4.9 n = 3	25.6 ± 3.8 n = 3
GGT	9.7 ± 5.1 n = 3	23 ± 9.9 n = 3
CREATI-NINE	0.76 ± 0.18 n = 3	0.73 ± 0.03 n = 3
AMYLASE	306 ± 52 n = 3	233 ± 36 n = 3
SODIUM	139.3 ± 0.9 n = 3	142 ± 2.0 n = 3
POTASS-IUM	7.0 ± 1.5 n = 3	5.5 ± 0.3 n = 3

[a] = Units (mean ± S.E.M) for the values are: Lactate dehydrogenase, IU/L; creatine phosphokinase, IU/L; blood urea nitrogen, mg/DL; calcium, mg/DL; glucose, mg/DL; phosphorus, mg/DL; total protein, g/DL; albumin, g/DL; globulin, g/DL; albumin/globulin ratio; cholesterol, mg/DL; total bilirubin, mg/DL; alkaline phosphatase, IU/L; serum glutamic pyruvic transaminase, IU/L; gamma glutamyl transpeptidase, IU/L; creatinine, mg/DL; amylase, IU/L; sodium, mEq/L; potassium, mEq/L.

Table 5. Laboratory Values[a] From Female Rabbits Receiving a Minimum Lethal Dose of Ricin.

	TIME (IN HOURS) AFTER INJECTION			
	0	12	24	36
LDH	78 ± 21.0 n = 3	340 ± 178 n = 5	1379 ± 283 n = 6	7590 n = 1
CPK	463 ± 78.9 n = 3	3576 ± 1286 n = 5	3900 ± 1249 n = 6	7795 n = 1
BUN	24 ± 1.3 n = 3	23 ± 1.8 n = 5	30 ± 2.4 n = 6	46 n = 1
CALCIUM	15.5 ± 0.2 n = 3	13.0 ± 0.7 n = 5	11.0 ± 0.25 n = 6	11.5 n = 1
GLUCOSE	188 ± 7.8 n = 3	142 ± 3.2 n = 5	144 ± 4 n = 6	71 n = 1
PHOS-PHORUS	5.5 ± 0.23 n = 3	5.6 ± 0.5 n = 5	6.45 ± 0.7 n = 6	7.1 n = 1
TOTAL PROTEIN	5.9 ± 0.26 n = 3	5.6 ± 0.1 n = 5	6.0 ± 0.2 n = 6	5.8 n = 1
ALBUMIN	4.0 ± 0.12 n = 3	4.1 ± 0.1 n = 5	3.8 ± 0.05 n = 6	3.7 n = 1
GLOBU-LIN	1.9 ± 0.17 n = 3	2.4 ± 0.4 n = 5	2.2 ± 0.2 n = 6	2.1 n = 1
A/G RATIO	2.2 ± 0.14 n = 3	2.2 ± 0.16 n = 5	1.8 ± 0.15 n = 6	1.8 n = 1
CHOLES-TEROL	71 ± 8.7 n = 3	78 ± 16.1 n = 5	115 ± 20.0 n = 6	89 n = 1
TOTAL BILIRU	0.15 ± 0.05 n = 3	0.28 ± 0.08 n = 5	1.9 ± 0.8 n = 6	1.6 n = 1
ALK PHOS	85 ± 15.5 n = 3	92 ± 11.7 n = 5	117 ± 26 n = 6	184 n = 1

Table 5 (cont'd). Laboratory Values[a] From Female Rabbits Receiving a Minimum Lethal Dose of Ricin.

	TIME (IN HOURS) AFTER INJECTION			
	0	12	24	36
SGPT (ALT)	49 ± 5.9 n = 3	71 ± 9.0 n = 5	456 ± 26 n = 6	1285 n = 1
GGT	5.3 ± 1.2 n = 3	3.6 ± 0.8 n = 5	9.5 ± 1.9 n = 6	32 n = 1
CREATI-NINE	1.5 ± 0.03 n = 3	1.3 ± 0.08 n = 5	1.6 ± 0.07 n = 6	1.9 n = 1
AMYLASE	280 ± 15.5 n = 3	230 ± 17 n = 5	242 ± 23.0 n = 6	349 n = 1
SODIUM	137 ± 0.33 n = 3	144 ± 5 n = 5	138 ± 0.7 n = 6	144 n = 1
POTASS-IUM	4.0 ± 0.4 n = 3	4.1 ± 0.15 n = 5	3.8 ± 0.11 n = 6	4.2 n = 1

[a] = Units (mean S.E.M) for the values are: Lactate dehydrogenase, IU/L; creatine phosphokinase, IU/L; blood urea nitrogen, mg/DL; calcium, mg/DL; glucose, mg/DL; phosphorus, mg/DL; total protein, g/DL; albumin, g/DL; globulin, g/DL; albumin/globulin ratio; cholesterol, mg/DL; total bilirubin, mg/DL; alkaline phosphatase, IU/L; serum glutamic pyruvic transaminase, IU/L; gamma glutamyl transpeptidase, IU/L; creatinine, mg/DL; amylase, IU/L; sodium, Meq/L; potassium, mEq/L.

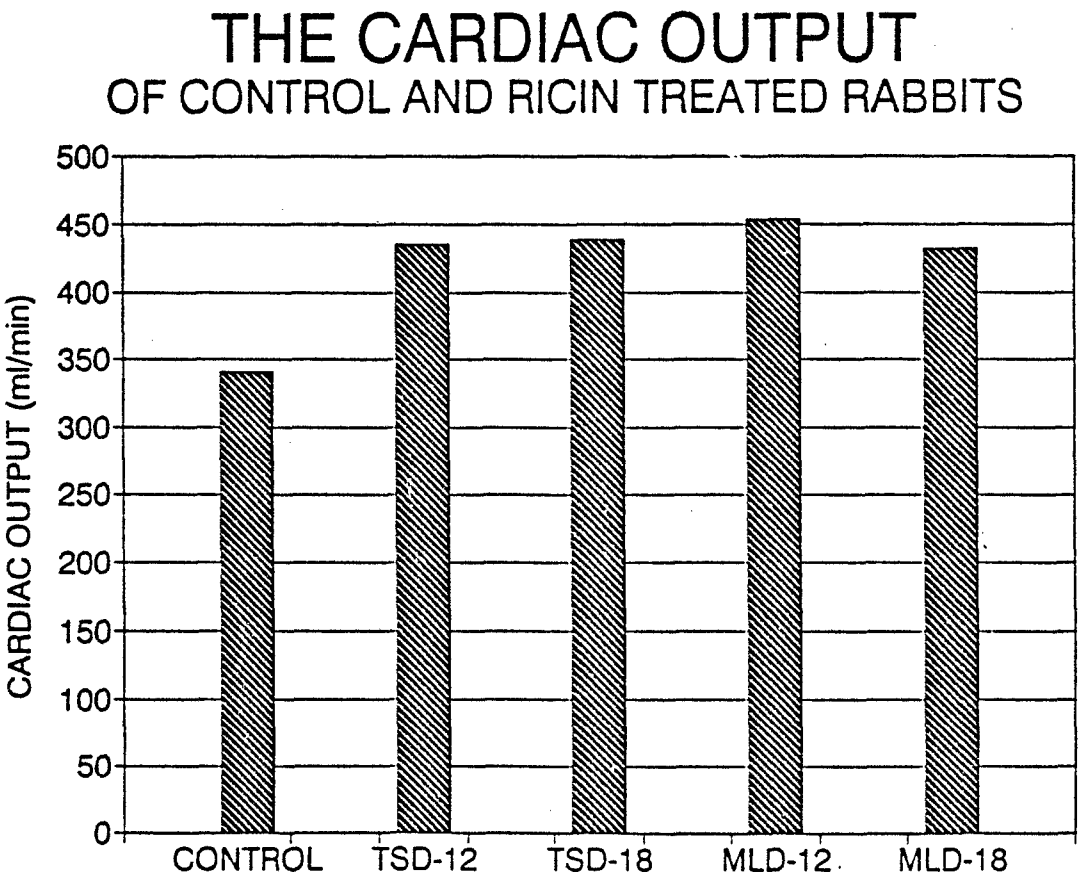

Figure 23. The cardiac output of control and ricin treated rabbits.
TSD = Toxic Sub-lethal Dose
MLD = Minimum Lethal Dose
-12 and -18 = Hours post-ricin i.v. injection.
n = 3 for TSD-18 hour values. In others, n = 2.

Table 6. The Effect of Ricin on Blood Flow ml/min per 100g Tissue[a].

	CONTROL	RICIN TREATED			
		TSD		MLD	
		12 hr	18 hr	12 hr	18 hr
SKIN	7	6	13	19	7
HEART	297	674	743	831	906
Inner	462	1050	1123	757	1128
Middle	421	819	1028	947	1363
Outer	426	866	1036	1014	1434
AORTA	19	22	38	33	29
LUNGS	70	88	204	72	33
TRACHEA	20	17	32	32	17
BRONCHII	23	26	62	57	30
FAT	36	28	80	37	20
LIVER	7	19	11	11	12
GALL BLADDER	113	128	197	277	236
SPLEEN	469	217	466	684	611
KIDNEY	434	641	460	594	673
Medullary	74	99	78	56	71
Cortex	882	1065	925	1052	1247
ADRENALS	139	435	231	244	219
MUSCLE[b]	11	6	11	14	18
TESTES	205	715	331	801	355
BRAIN	73	180	76	48	36
Cerebrum	75	168	76	44	34
Pituitary	493	543	290	476	727
Thalamus	54	160	66	63	35
Midbrain	85	206	78	30	40
Cerebellum	87	256	94	66	41

Pons	50	146	68	60	46
Medulla	52	142	50	42	29
GI TRACT[c]	60	58	83	77	77
Esophagus	28	24	30	45	25
STOMACH	63	51	143	103	97
Cardiac	22	67	32	31	27
Pylorus	60	21	150	129	109
Lessor curv.	44	82	104	86	98
Greater curv.	139	32	253	177	170
SM. INTESTINE	78	105	124	131	127
Duodenum	103	117	163	222	158
Upper jejunum	45	92	136	102	146
Lower jejunum	75	85	112	132	102
ILEUM	88	134	96	104	129
CECUM	76	61	78	67	95
L. INTESTINE	55	49	41	37	40

a. Measured by injection of radiolabeled microspheres into the left ventricle. n = 2 except for the TSD, 18hr group, where n = 3.
b. Gluteus maximus muscle.
c. The GI Tract did not include the appendix, cecum and rectum.

Table 7. The Effects of Ricin on the Percent of Total Cardiac Output Received by Organs[a].

	Control	RICIN TREATED			
		TSD		MLD	
		12 HR	18 HR	12 HR	18 HR
	% Output	% Output	% Output	% Output	% Output
Heart	3.69	6.53	6.95	7.75	8.44
Aorta	0.01	0.02	0.03	0.02	0.02
Lungs (total)	1.26	1.36	2.81	0.93	0.49
L. Lung	0.57	0.55	1.22	0.36	0.21
R. Lung	0.69	0.81	1.59	0.57	0.28
Trachea	0.03	0.02	0.05	0.04	0.02
Bronchii	0.01	0.01	0.03	0.03	0.01
Liver	1.37	2.91	1.49	1.74	2.04
Gall bladder	0.04	0.05	0.04	0.03	0.04
Spleen	0.88	0.50	0.74	0.81	1.05
Kidney	14.99	19.10	9.72	13.72	18.70
Adrenals	0.06	0.16	0.07	0.07	0.08
Muscle	3.03	1.56	3.13	4.74	5.09
Testes	0.02	0.01	0.02	0.02	0.02
Brain	1.79	3.27	1.38	0.99	0.64
GI Tract[b]	13.28	11.01	16.80	14.76	15.16
Esophagus	0.09	0.07	0.08	0.13	0.07
Stomach	3.41	2.23	5.73	4.07	4.31
Sm. intestine	7.66	7.42	9.75	9.29	9.38
L. intestine	1.69	1.08	0.97	0.95	1.00

a. Measured by injection of radiolabeled microspheres into the left ventricle. n = 2 except for the TSD, 18hr group, where n = 3.

b. The GI Tract did not include the appendix, cecum and rectum.

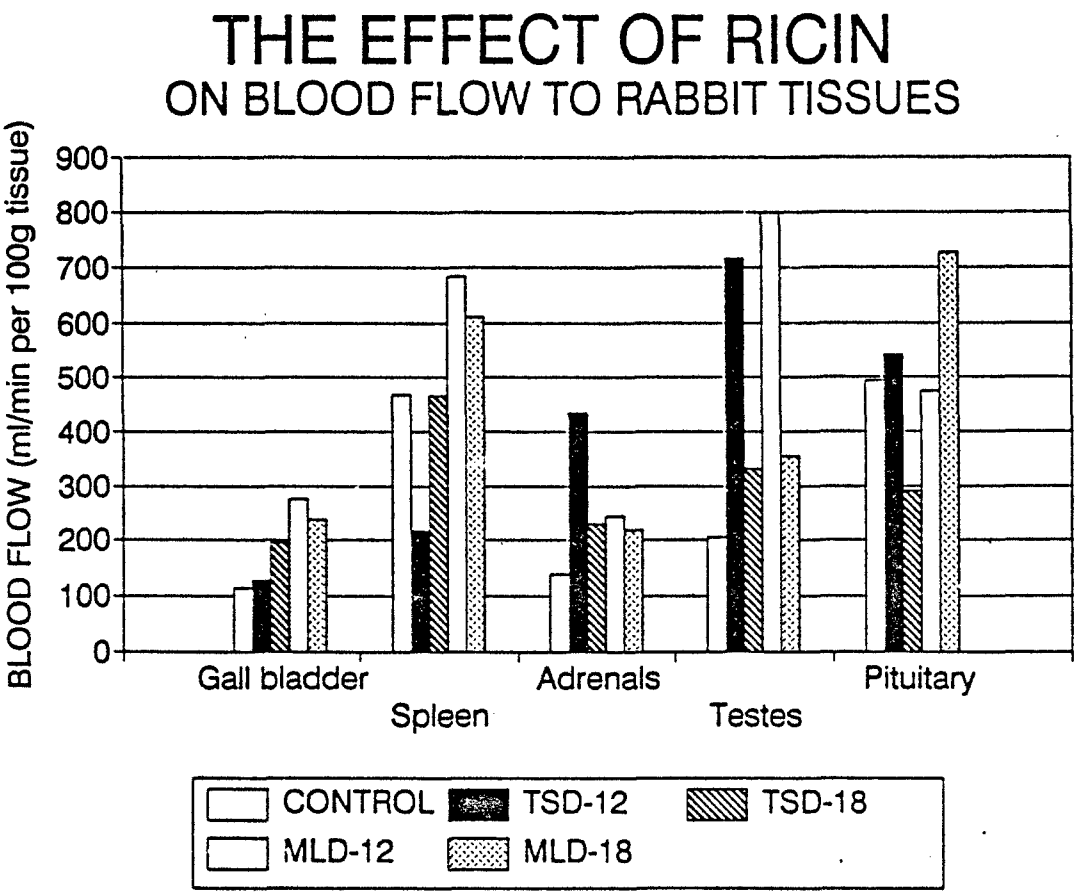

Figure 24. The effect of ricin on blood flow to the rabbit gall bladder, spleen, adrenals, testes, and pituitary.
Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

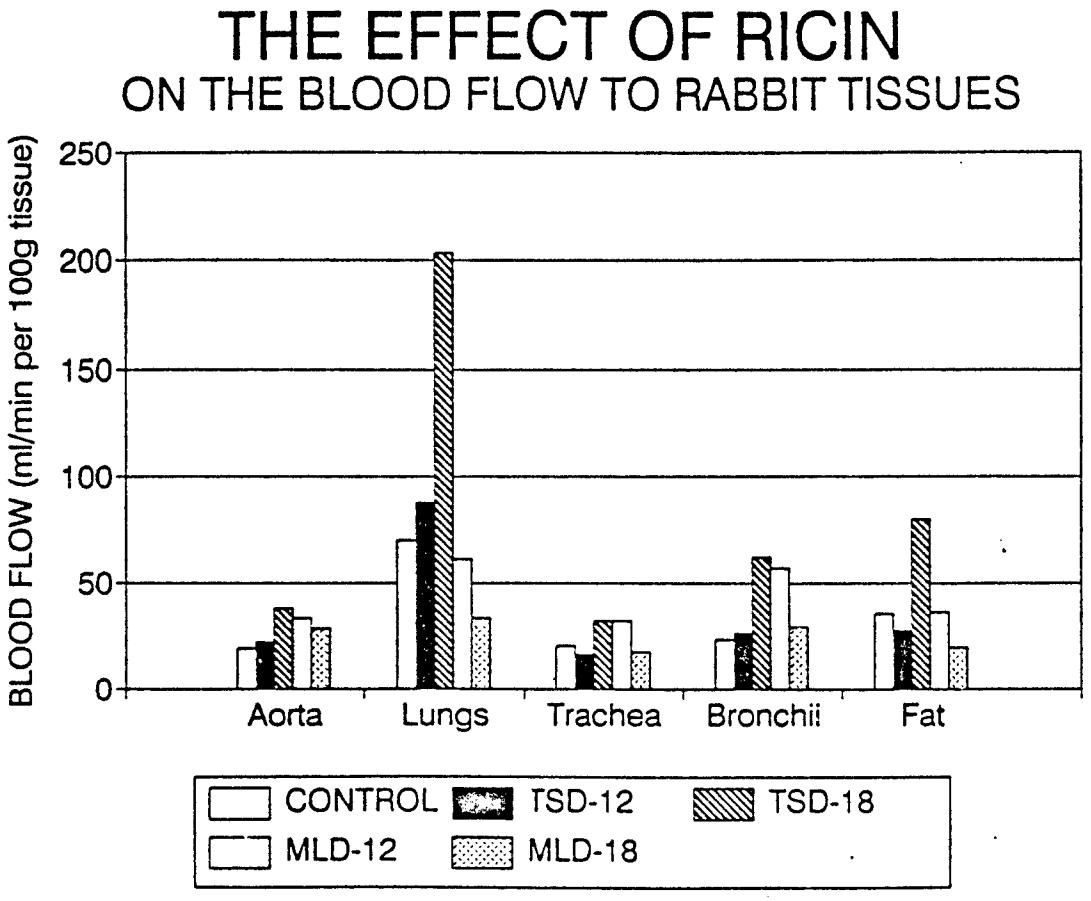

Figure 25. The effect of ricin on blood flow to the rabbit aorta, lungs, trachea, bronchial tree and fat.
Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

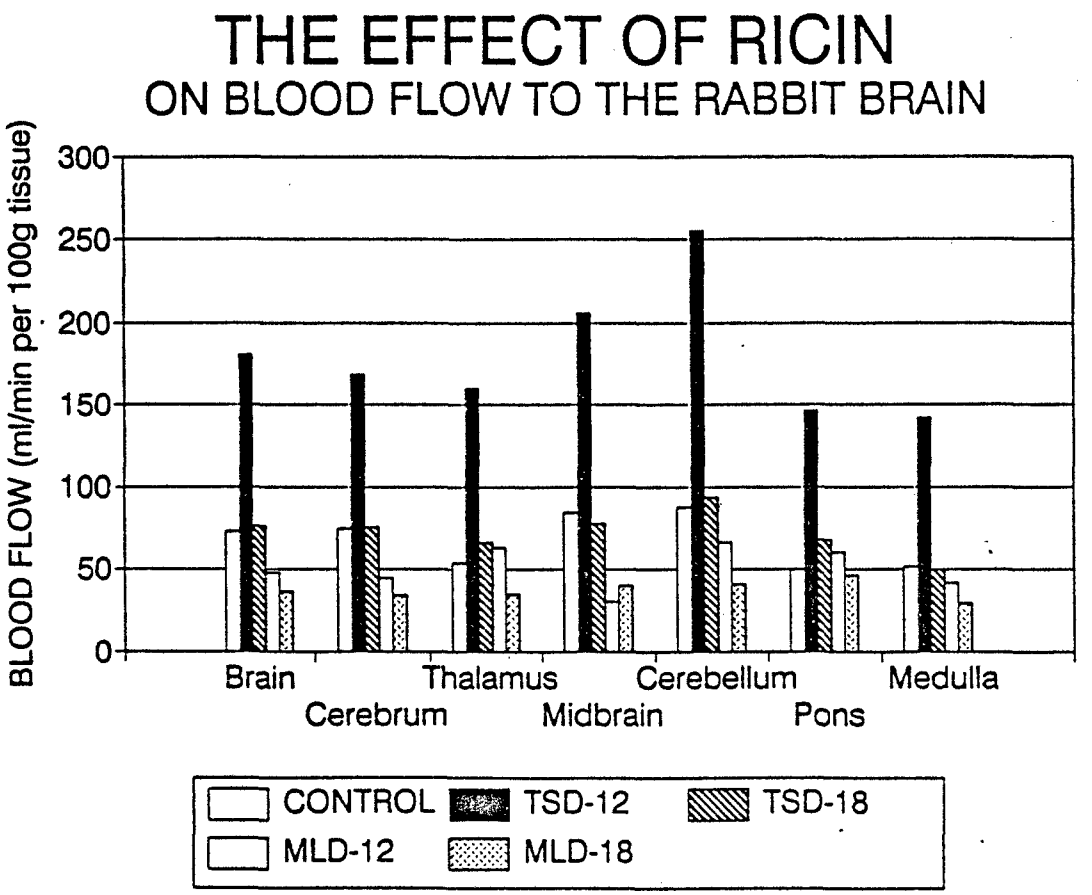

Figure 26. The effect of ricin on blood flow to the rabbit brain, cerebrum, thalamus, midbrain, cerebellum, pons and medulla.
Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

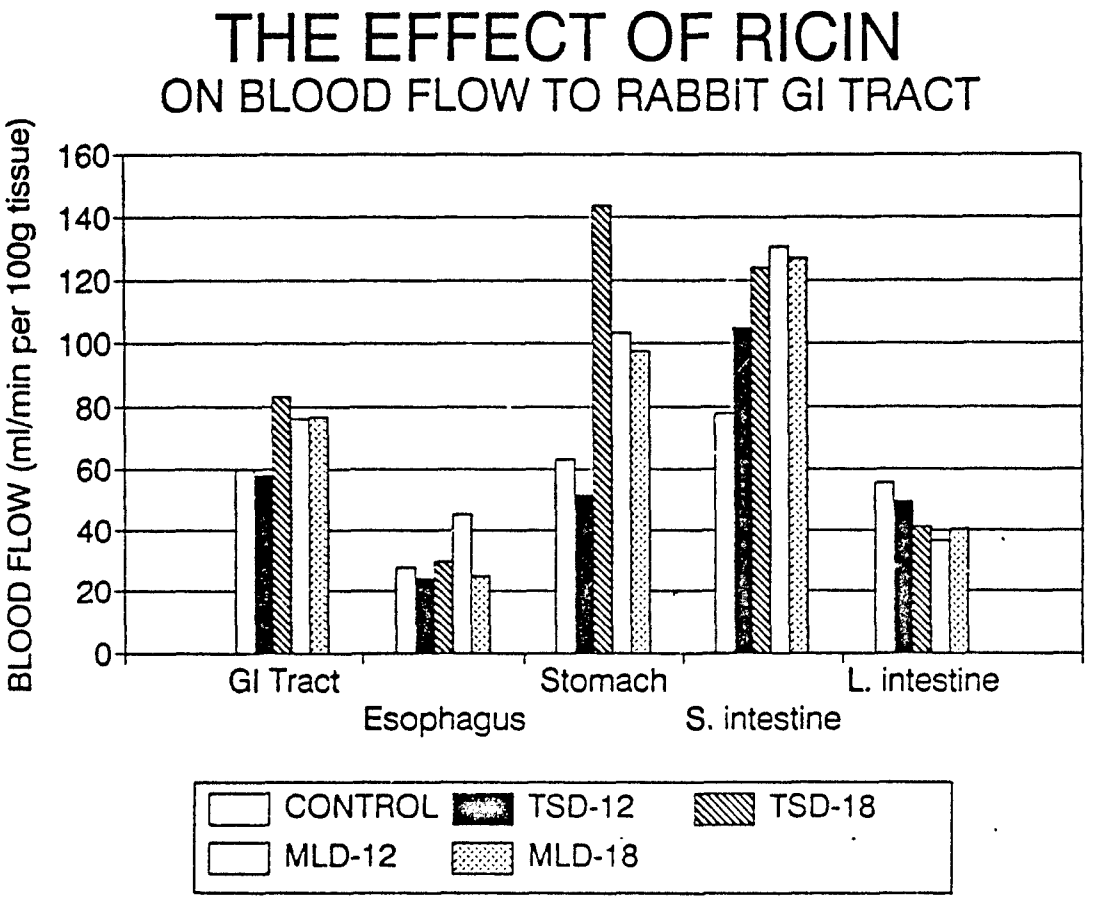

Figure 27. The effect of ricin on blood flow to the rabbit GI tract, esophagus, stomach, small intestine, and large intestine.
Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.
* Does not include the cecum, appendix, or rectum.

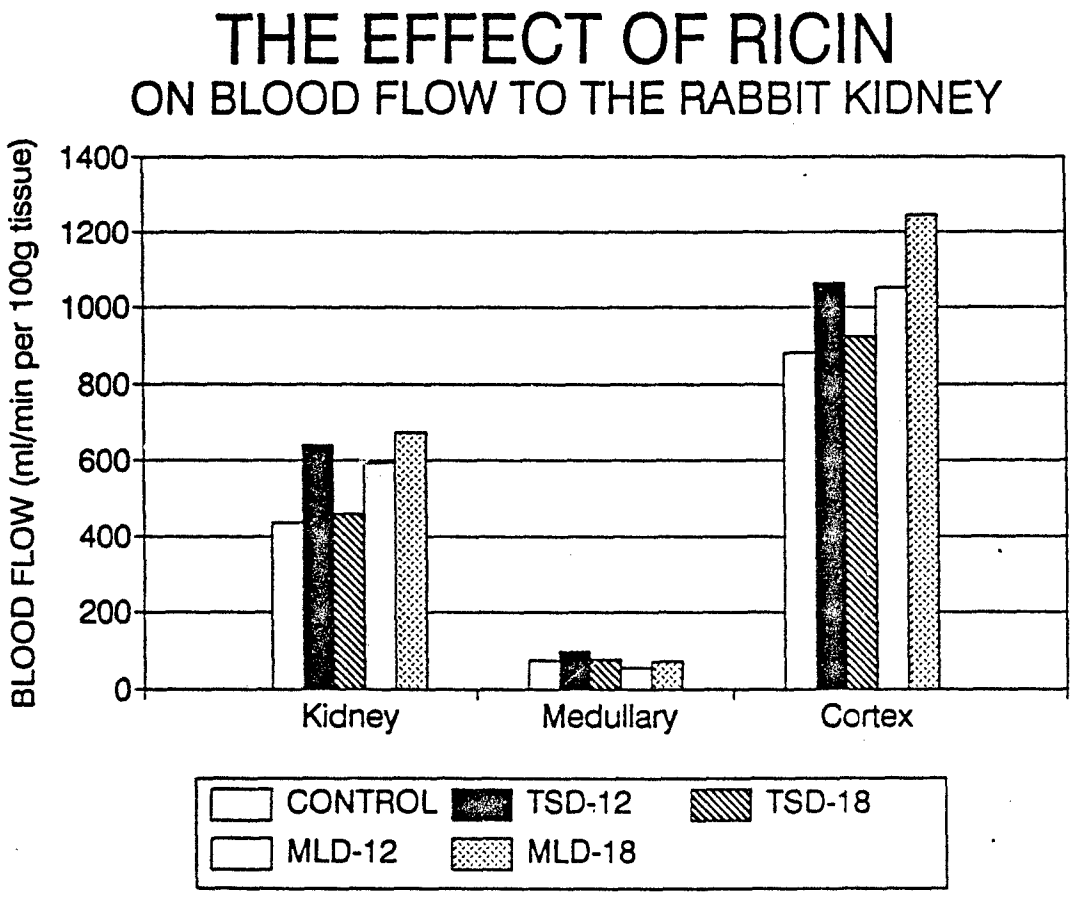

Figure 28. The effect of ricin on blood flow to the rabbit kidney.
Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

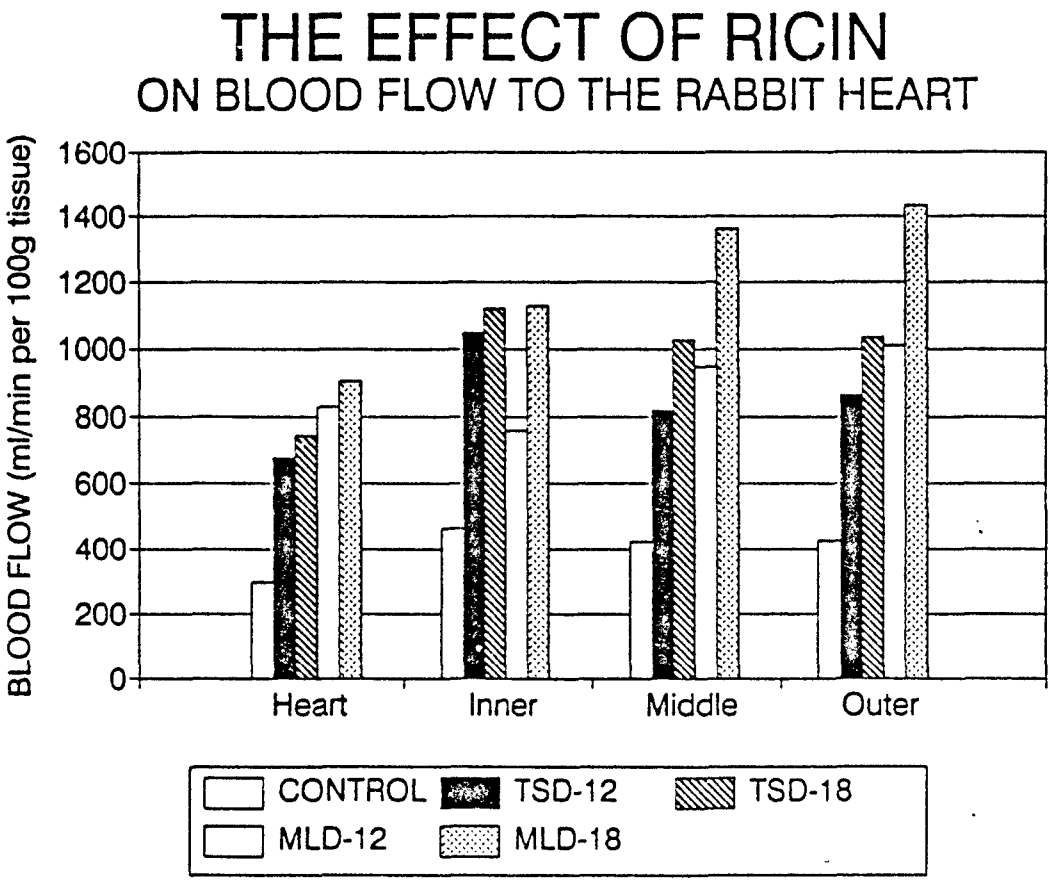

Figure 29. The effect of ricin on blood flow to the rabbit heart.
Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

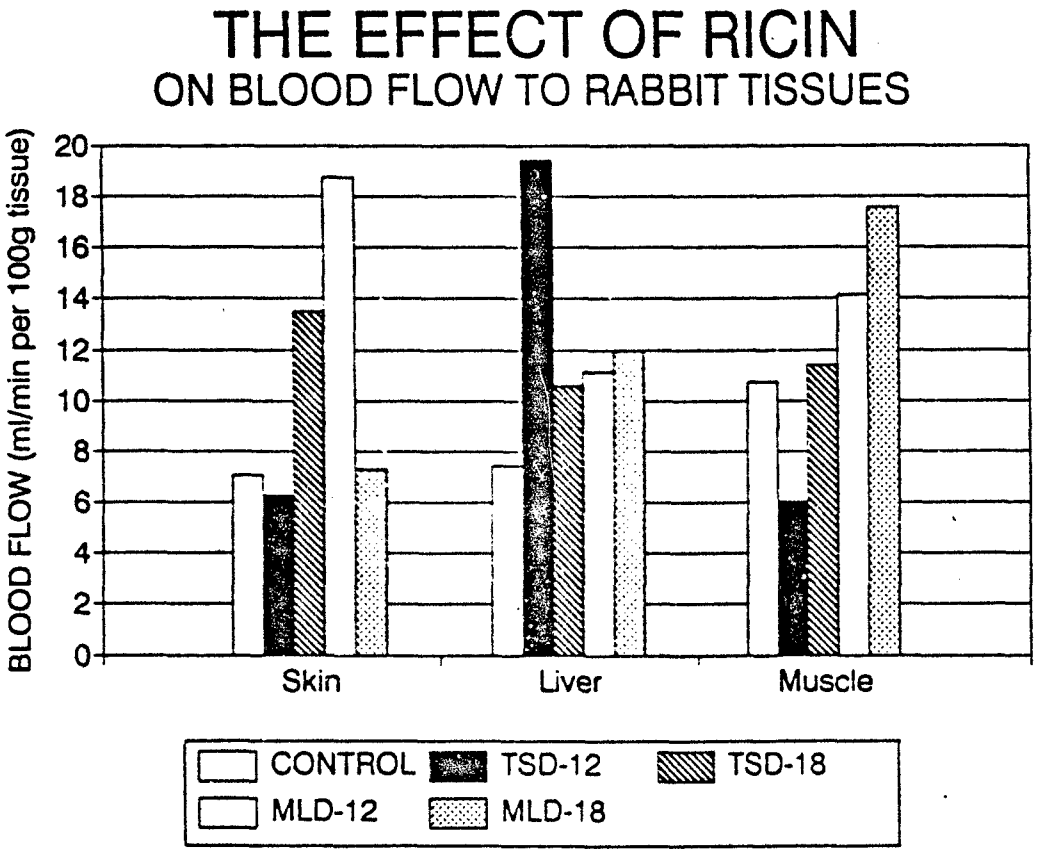

Figure 30. The effect of ricin on blood flow to the rabbit skin, liver, and muscle. Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

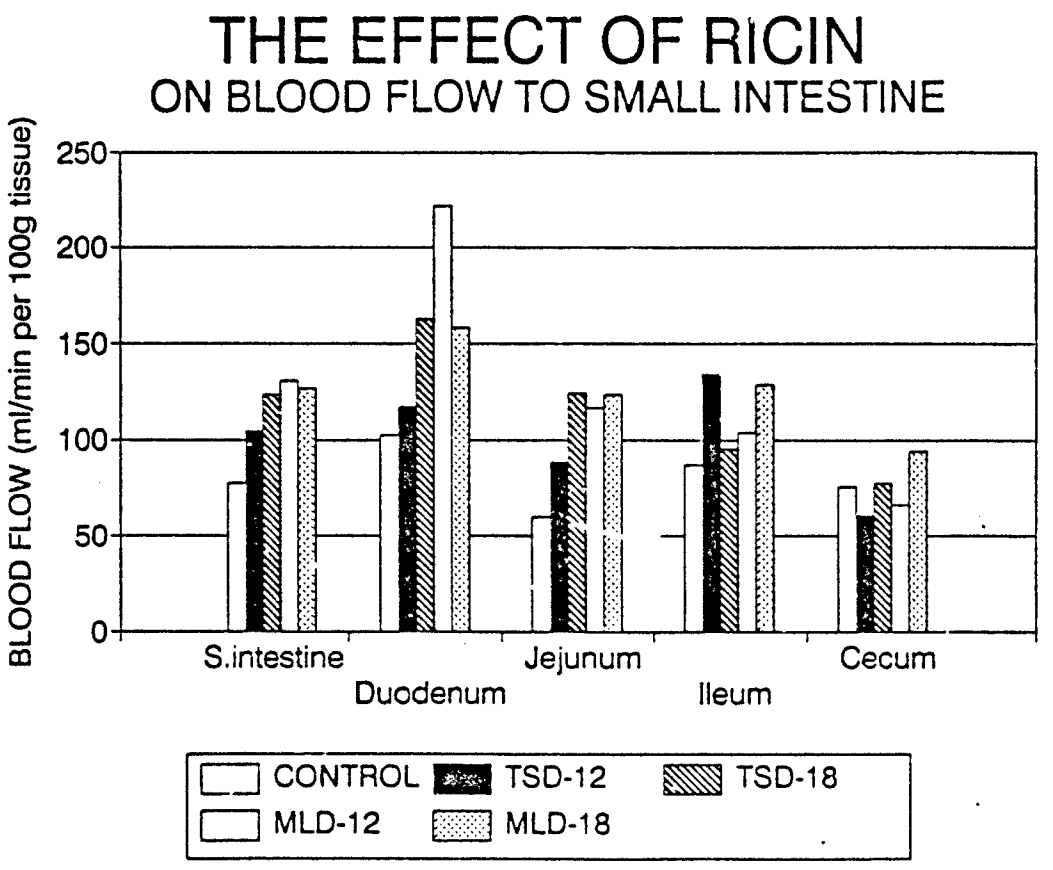

Figure 31. The effect of ricin on blood flow to the rabbit small intestine and cecum. Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

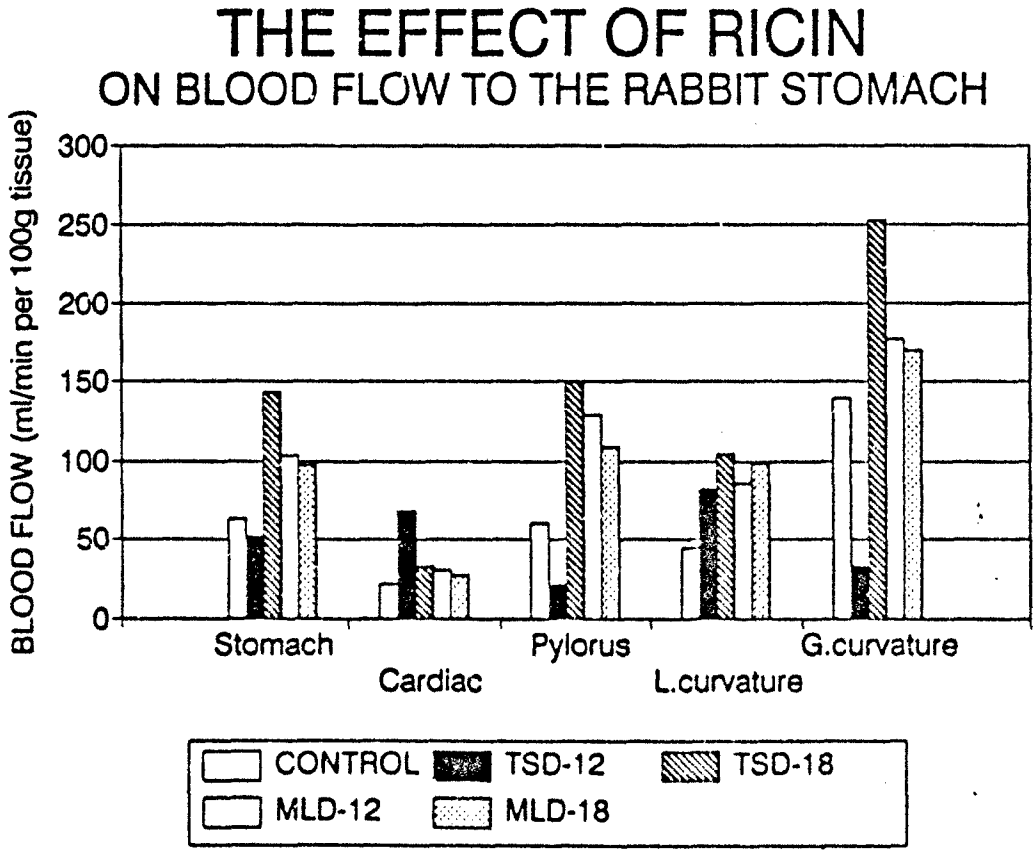

Figure 32. The effect of ricin on blood flow to the rabbit stomach.
Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

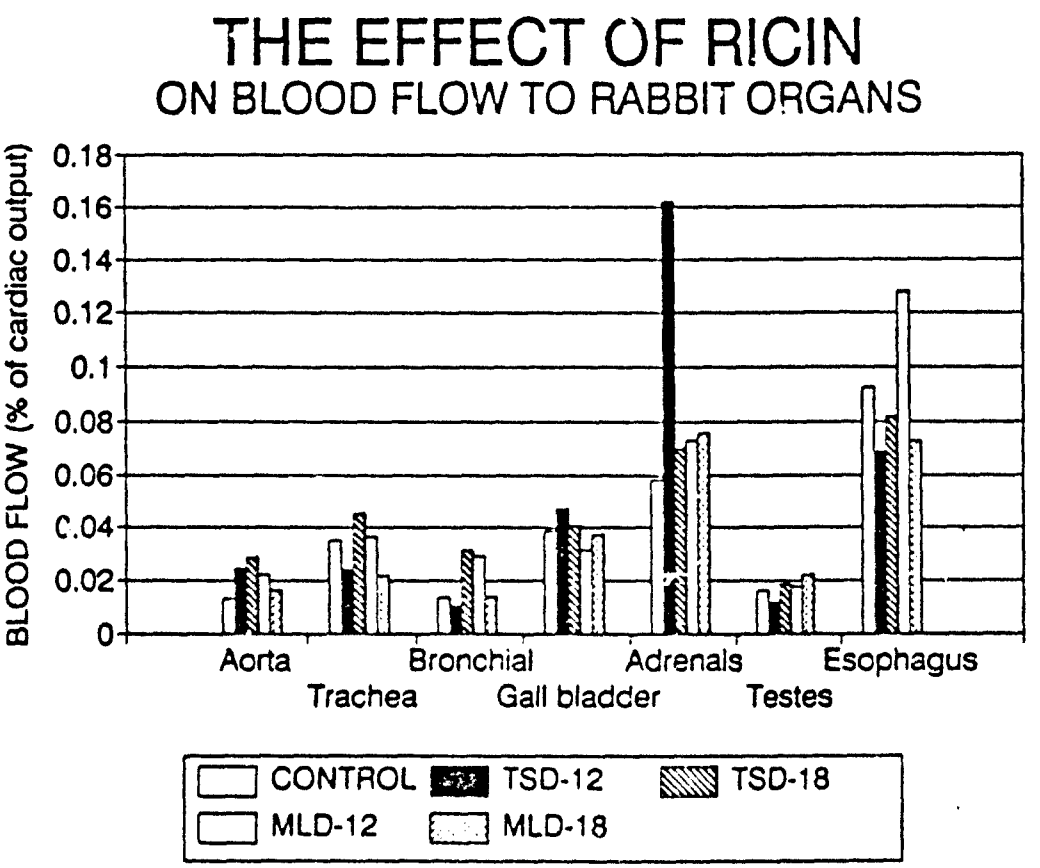

Figure 33. The effect of ricin on the percent of cardiac output received by the rabbit aorta, trachea, gall bladder, adrenals, testes and esophagus. Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

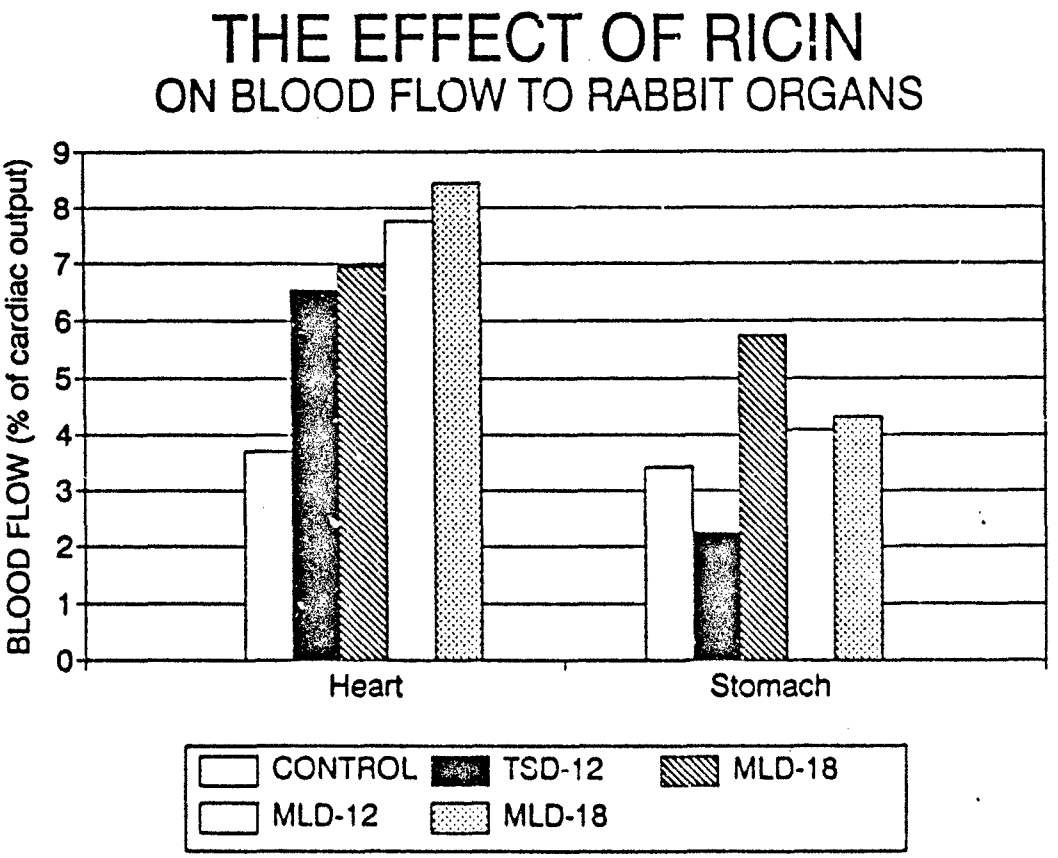

Figure 34. The effect of ricin on the percent of cardiac output received by the rabbit heart and stomach.
Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

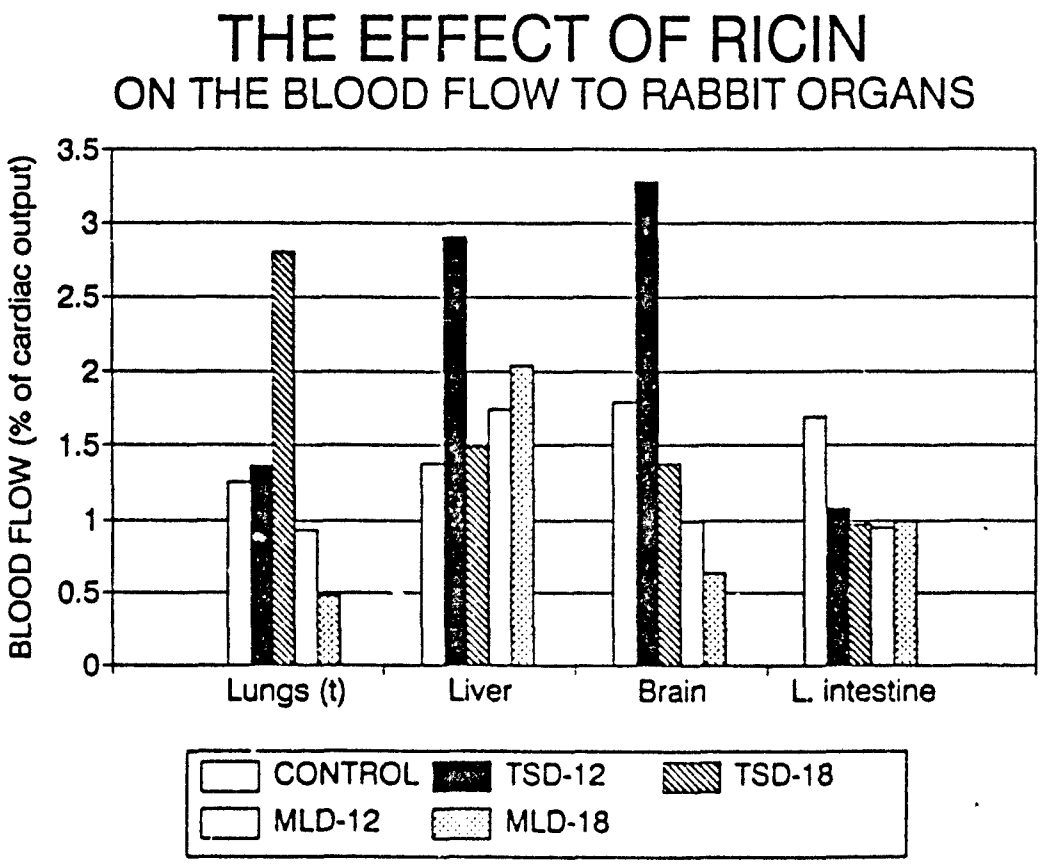

Figure 35. The effect of ricin on the percent of cardiac output received by the rabbit lungs, liver, brain and large intestine.
Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

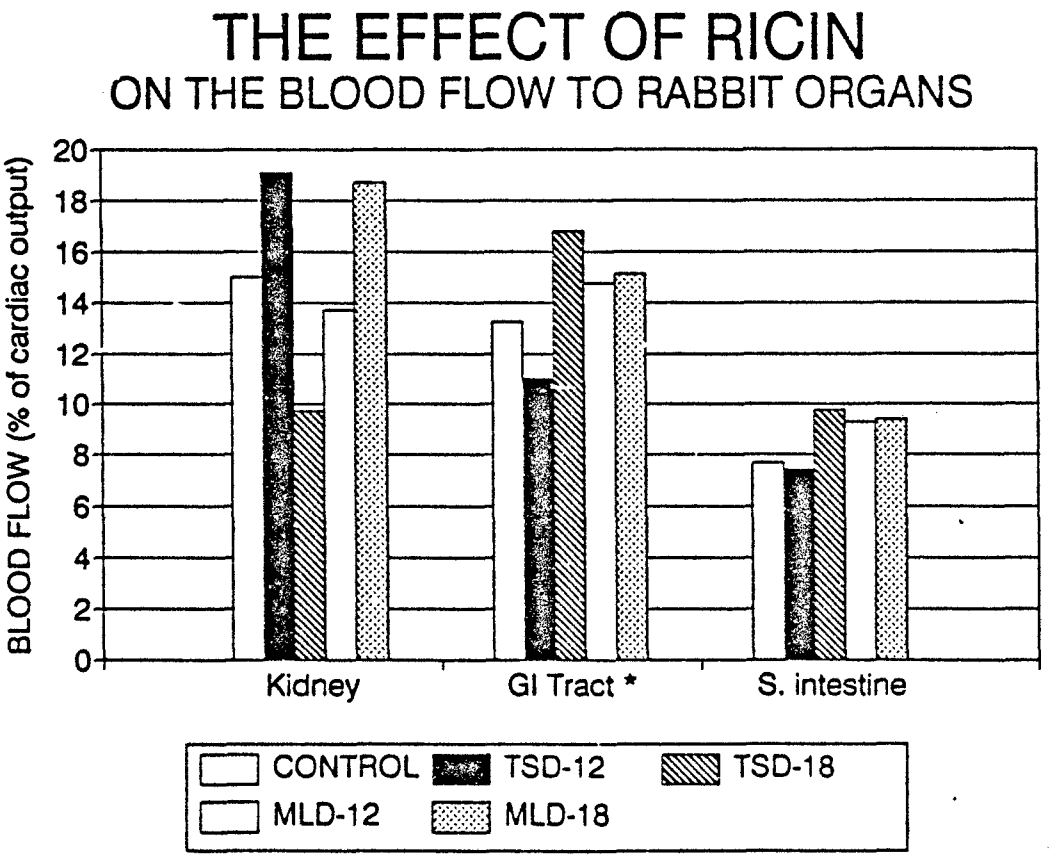

Figure 36. The effect of ricin on the percent of cardiac output received by the rabbit kidney, GI tract, and small intestine.
* Does not include the appendix, cecum, and rectum.
Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

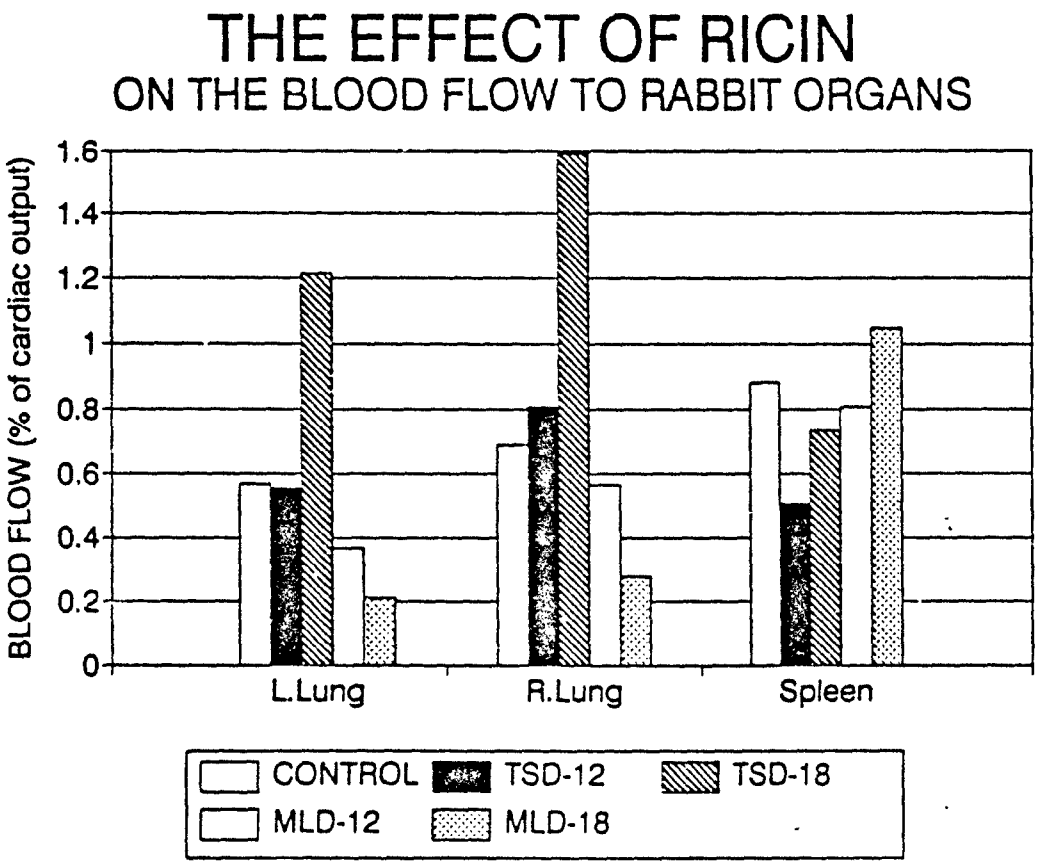

Figure 37. The effect of ricin on the percent of cardiac output received by the rabbit left lung, right lung and spleen.
Symbols same as in Figure 23.
n = 3 for TSD-18 hour values. In others, n = 2.

shunts, or have been delivered to the lungs through a branch of the brachial artery. In whatever case, a toxic sub-lethal dose of ricin slightly increased blood flow to the lungs at 12 hours, and greatly increased it at 18 hours. On the other hand, a minimum lethal dose of ricin did not alter blood flow to the lungs at 12 hours, but did so at 18 hours to a much greater degree, reducing it to 39% of control.

Blood flow to the left and right kidneys were very similar (Figure 38) and thus we can assume adequate mixing of microspheres and blood.

G. Effects of Ricin Administration to Rabbits on Contractions and Relaxations of the Helically-Cut Central Ear Artery to Agonists.

1. To Norepinephrine

The responses of the central ear artery to norepinephrine (NE) are shown in Figures 39 to 44. The values which are plotted are shown in Tables 8 and 9. The EC_{50} (a measure of sensitivity) and the maximal contraction to NE, are shown in Table 10. Compared to control, ricin treatment typically increases the maximal contraction. However, none of the treated groups were significantly different from control ($p > 0.05$). Ricin treatment also typically increased the EC_{50} to NE which indicates a decrease in the sensitivity of the tissue to NE. The EC_{50} for the rabbit central ear arteries in the 18 hr minimum lethal dose group was significantly different from control at $p = 0.054$.

2. To Tyramine and KCl

The contractions of the central ear artery to KCl and tyramine are shown in Tables 8 and 9. There was no significant difference between control and ricin treated groups ($p > 0.05$). This indicates that the contractile mechanism and the ability of the tissue to release NE to a pharmacological stimuli, bind to a receptor and cause a response is not altered by ricin.

3. To Papaverine

The ability of papaverine to relax the central ear artery maximally contracted with NE is shown in Tables 8 and 9. There is no significant difference in the relaxation to papaverine between control and ricin treated rabbits ($p > 0.05$).

4. Comparisons of the Effects of Vasoactive Compounds

Ratios of NE:K^+, Tyr:NE and Tyr:K^+ (Table 11) indicate that there is no major impairment in the pharmacomechanical coupling system of the α-adrenergic receptor. With the miniumum lethal dose of ricin at 18 hours post injection there does seem to be a decrease in the ratios, indicating a slight impairment in the coupling system. The Papavarine:NE ratio is unchanged with ricin treatment indicating that there is no change in the ability of the artery to relax.

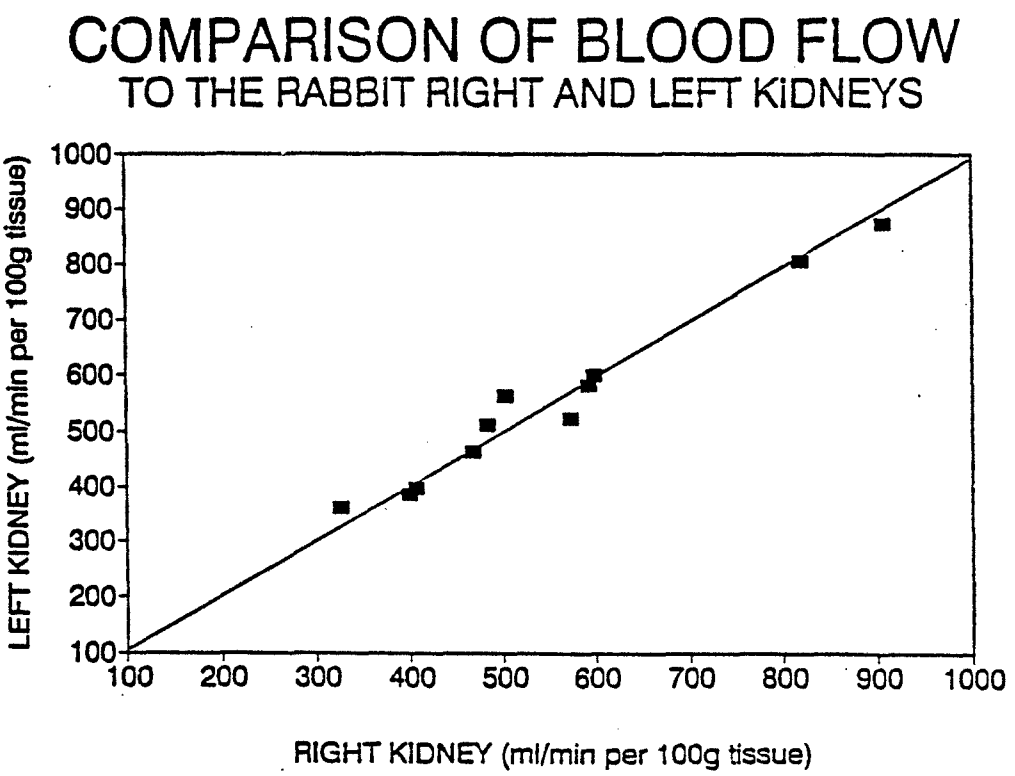

Figure 38. Comparison of blood flow to the rabbit right and left kidney as determined by radioactive microspheres in all of the rabbits studied.

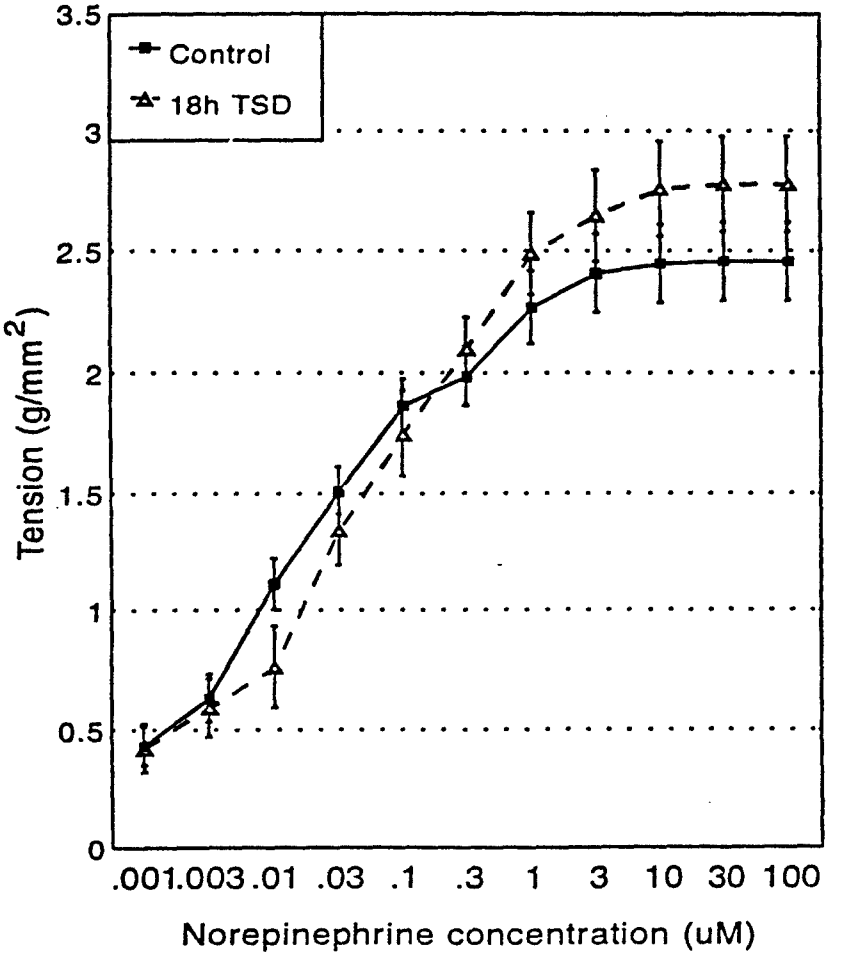

Figure 39. Response of rabbit central ear artery strips to norepinephrine 18 hours after i.v. injection of a toxic sub-lethal dose of ricin. Each point is the mean ± SEM of 18-46 strips from 6-12 rabbits.

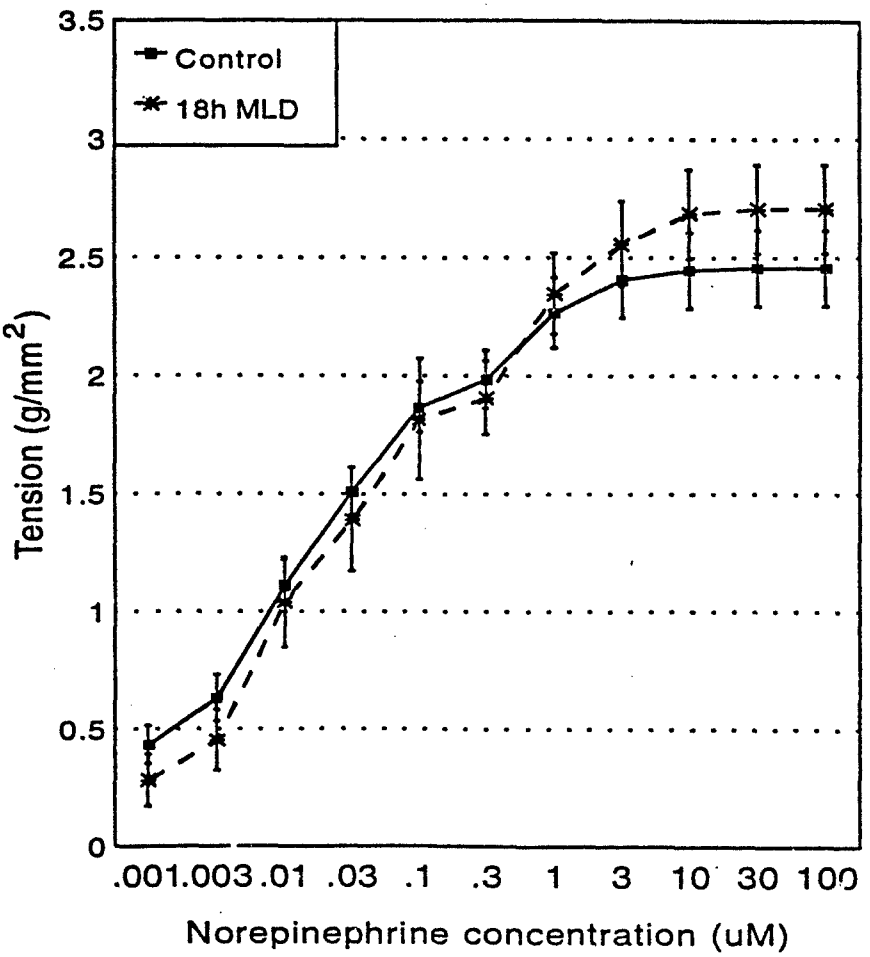

Figure 40. Response of rabbit central ear artery strips to norepinephrine 18 hours after i.v. injection of a minimal lethal dose of ricin. Each point is the mean ± SEM of 18-39 strips from 6-12 rabbits.

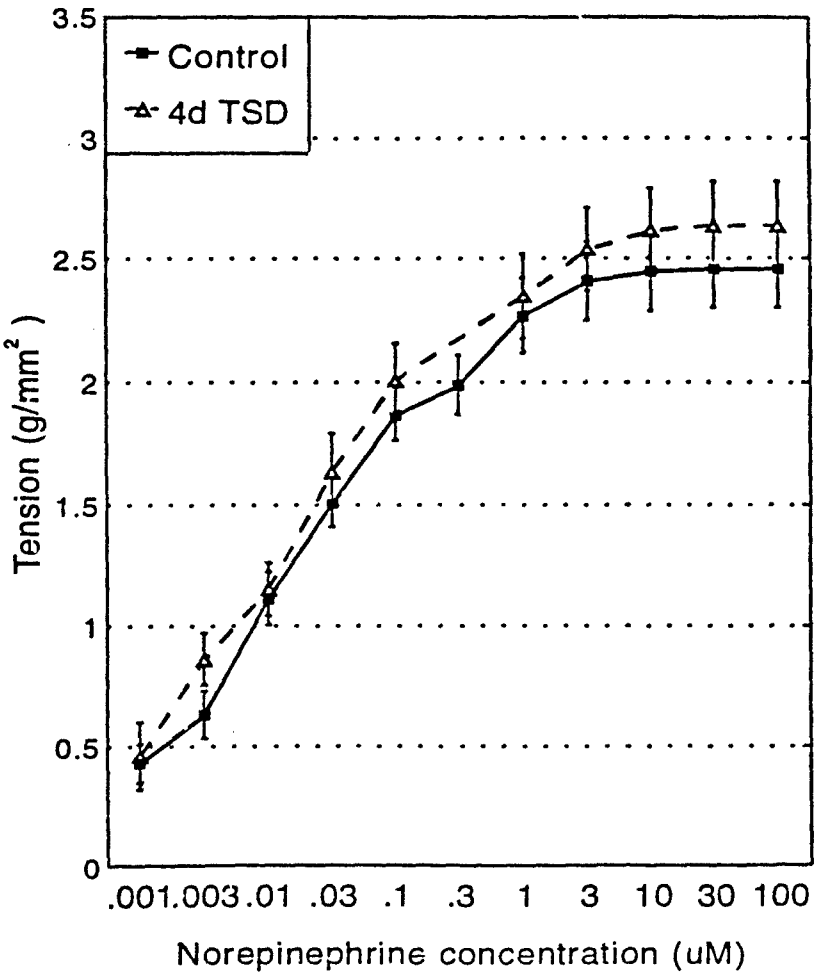

Figure 41. Response of rabbit central ear artery strips to norepinephrine 4 days after i.v. injection of a toxic sub-lethal dose of ricin. Each point is the mean ± SEM of 18-47 strips from 6-12 rabbits.

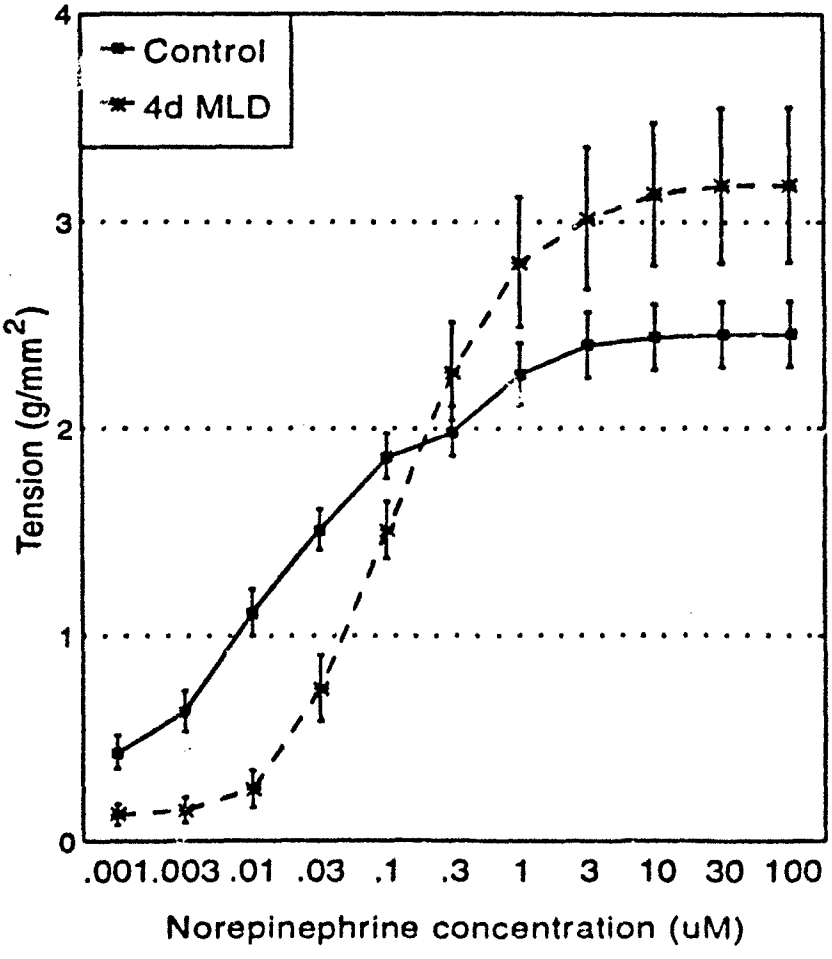

Figure 42. Response of helically cut rabbit central ear artery strips to norepinephrine 4 days after i.v. injection of a minimal lethal dose of ricin. Each point is the mean ± SEM of 6-39 strips from 6-12 rabbits.

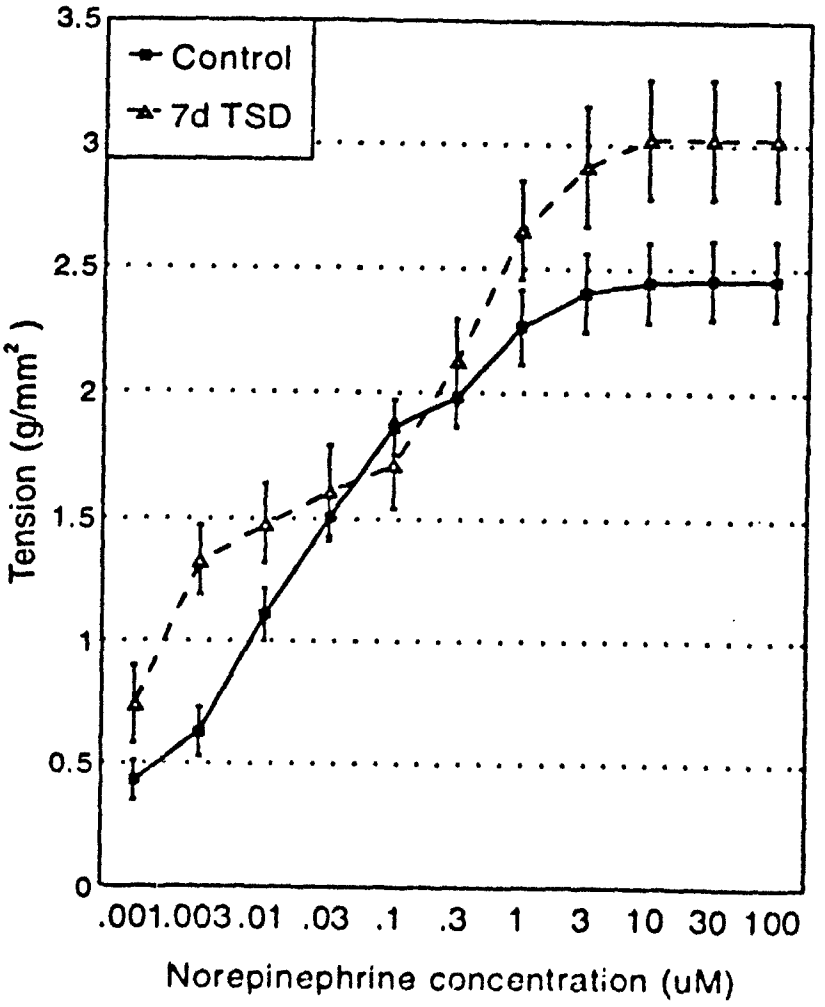

Figure 43. Response of rabbit central ear artery strips to norepinephrine 7 days after i.v. injection of a toxic sub-lethal dose of ricin. Each point is the mean ± SEM of 17-39 strips from 6-12 rabbits.

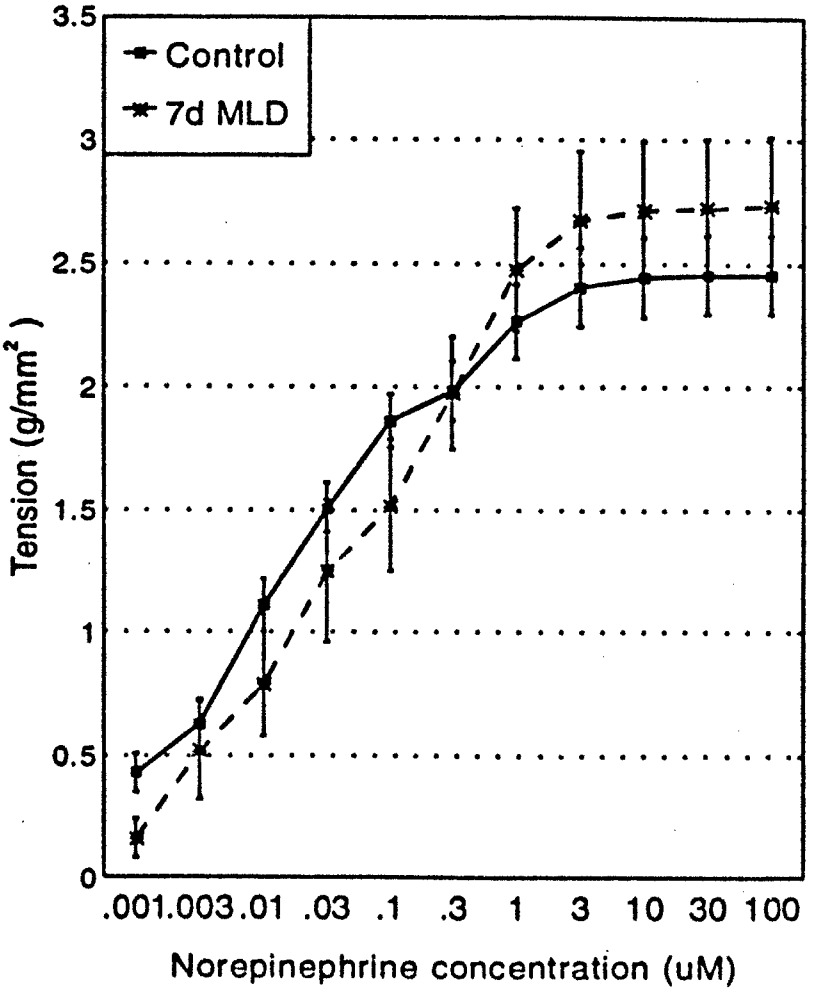

Figure 44. Response of rabbit central ear artery strips to norepinephrine 7 days after i.v. injection of a minimal lethal dose of ricin. Each point is the mean ± SEM of 12-39 strips from 6-12 rabbits.

Table 8. Contractions[a] of Central Ear Arteries from Rabbits Given a Toxic Sub-lethal Dose of Ricin i.v, to Added Agents.

AGENTS	CONTROL	Time Period From Ricin Administration to Obtaining Tissues		
		18 HOURS	4 DAYS	7 DAYS
	Mean±SEM(n)	Mean±SEM(n)	Mean±SEM(n)	Mean±SEM(n)
KCl, 120 Mm	1.81±0.11(39)	2.05±0.15 (46)	1.87±0.13 (46)	2.26±0.18 (31)
Tyramine, 100 μM	1.73±0.24(16)	2.03±0.17 (28)	1.60±0.18 (29)	2.22±0.26 (14)
NE,0.001μM	0.43±0.08 (23)	0.42±0.10 (18)	0.46±0.14 (18)	0.74±0.16 (17)
0.003 μM	0.63±0.10 (23)	0.59±0.12 (18)	0.86±0.11 (18)	1.33±0.14 (17)
0.01 μM	1.11±0.11 (23)	0.76±0.17 (18)	1.15±0.11 (18)	1.48±0.16 (17)
0.03 μM	1.51±0.10 (23)	1.34±0.15 (18)	1.64±0.15 (18)	1.61±0.18 (17)
0.1 μM	1.87±0.11 (23)	1.75±0.18 (18)	2.01±0.15 (18)	1.72±0.18 (17)
0.3 μM	1.99±0.12 (39)	2.10±0.13 (46)	1.99±0.15 (47)	2.13±0.17 (31)
1 μM	2.27±0.15 (39)	2.49±0.17 (46)	2.35±0.17 (47)	2.66±0.20 (31)
3 μM	2.41±0.16 (39)	2.65±0.19 (46)	2.54±0.17 (47)	2.92±0.24 (31)
10 μM	2.45±0.16 (39)	2.76±0.20 (46)	2.62±0.17 (47)	3.03±0.24 (31)
30 μM	2.46±0.16 (39)	2.78±0.20 (46)	2.64±0.18 (47)	3.03±0.24 (31)
100 μM	2.46±0.16 (39)	2.78±0.20 (46)	2.64±0.18 (47)	3.03±0.24 (31)
Papaverine, 100 μM	-2.63±0.43(16)	-3.12±0.31(28)	-2.95±0.27 (24)	-2.98±0.27(14)

a. Stated as g tension/mm^2. Negative numbers (to papaverine) denote relaxation from the peak NE contraction. n=the number of artery strips, from at least 6 rabbits.

Table 9. Contractions[a] of Central Ear Arteries from Rabbits Given a Minimum Lethal Dose of Ricin i.v., to Added Agents.

AGENTS	CONTROL	Time Period From Ricin Administration to Obtaining Tissues		
		18 HOURS	4 DAYS	7 DAYS
	Mean±SEM(n)	Mean±SEM(n)	Mean±SEM(n)	Mean±SEM(n)
KCl, 120 mM	1.81±0.11(39)	2.14±0.18 (37)	2.30±0.25 (34)	1.99±0.18 (27)
Tyramine, 100 μM	1.73±0.24(16)	1.46±0.11 (20)	2.48±0.36 (19)	2.03±0.26 (12)
NE,0.001μM	0.43±0.08 (23)	0.28±0.11 (18)	0.13±0.05 (6)	0.16±0.08 (12)
0.003 μM	0.63±0.10 (23)	0.45±0.13 (18)	0.15±0.06 (6)	0.52±0.20 (12)
0.01 μM	1.11±0.11 (23)	1.04±0.19 (18)	0.25±0.09 (6)	0.79±0.21 (12)
0.03 μM	1.51±0.10 (23)	1.39±0.22 (18)	0.74±0.16 (6)	1.25±0.29 (12)
0.1 μM	1.87±0.11 (23)	1.82±0.26 (18)	1.51±0.14 (6)	1.52±0.27 (12)
0.3 μM	1.99±0.12 (39)	1.91±0.16 (38)	2.28±0.24 (25)	1.98±0.23 (24)
1 μM	2.27±0.15 (39)	2.35±0.17 (38)	2.81±0.31 (25)	2.48±0.25 (24)
3 μM	2.41±0.16 (39)	2.56±0.18 (38)	3.02±0.34 (25)	2.68±0.28 (24)
10 μM	2.45±0.16 (39)	2.69±0.19 (38)	3.14±0.34 (25)	2.72±0.28 (24)
30 μM	2.46±0.16 (39)	2.71±0.19 (38)	3.18±0.37 (25)	2.73±0.28 (24)
100 μM	2.46±0.16 (39)	2.71±0.19 (38)	3.18±0.37 (25)	2.74±0.28 (24)
Papaverine, 100 μM	-2.63±0.43(16)	-2.72±0.17(20)	-3.31±0.66 (19)	-3.11±0.36(12)

a. Stated as g tension/mm². Negative numbers (to papaverine) denote relaxation from the peak NE contraction. n=number of artery strip, from at least 6 rabbits.

Table 10. The EC_{50} for Norepinephrine (NE) and Maximal Contraction to Norepinephrine of Central Ear Artery Strips from Rabbits Receiving Ricin.

TREATMENT	EC_{50} to NE (nM) Mean ± SEM[a] (n)	Maximal tension to NE (g) Mean ± SEM[a] (n)
Control	16 ± 2 (23)	2.46 ± 0.16 (39)
Ricin, a toxic sub-lethal dose 18 h	28 ± 8 (17)	2.78 ± 0.20 (46)
4 d	21 ± 5 (18)	2.64 ± 0.18 (47)
7 d	16 ± 8 (16)	3.03 ± 0.24 (31)
Ricin, a minimum lethal dose 18 h	60 ± 28 (16)	2.71 ± 0.19 (38)
4 d	46 ± 8 (15)	2.77 ± 0.30 (34)
7 d	39 ± 19 (16)	2.64 ± 0.25 (27)

[a] n=number of artery strips. Number of rabbits = 4-9

Table 11. Ratios of Mean Contractions of 0.3 μM Norepinephrine (NE) to that of 120 mM Potassium and of Tyramine (Tyr) to 0.3 μM Norepinephrine and Papaverine to 100 μM NE in Central Ear Arteries from Rabbits Given a Toxic Sub-lethal Dose or a Minimum Lethal Dose of Ricin i.v.

AGENTS		Time Period Between Ricin Administration and Removing Artery		
	CONTROL	18 HOURS	4 DAYS	7 DAYS
	Mean	Mean	Mean	Mean
RICIN a toxic sub-lethal dose				
$\frac{NE}{K^+}$	1.10	1.02	1.06	0.94
$\frac{Tyr}{NE}$	0.94	0.94	0.92	0.95
$\frac{Tyr}{K^+}$	0.82	0.89	0.85	0.79
$\frac{Papav.}{NE}$	1.02	1.01	1.17	0.84
RICIN a minimum lethal dose				
$\frac{NE}{K^+}$	1.10	0.89	0.99	0.99
$\frac{Tyr}{NE}$	0.94	0.85	1.00	0.83
$\frac{Tyr}{K^+}$	0.82	0.66	0.85	0.79
$\frac{Papav.}{NE}$	1.02	1.04	1.06	0.89

n = 24-46 strips from 6-12 rabbits.

H. Effects of Ricin Administration on the Relaxation of Norepinephrine Contracted Aorta Rings to Relaxant Compounds.

1. To Methacholine

The responses of aorta rings to methacholine are shown in Figures 45 to 50. The maximum relaxation was increased at 18 hours following toxic sub-lethal and minimum lethal doses of ricin. The relaxation returned toward control at 4 days and by 7 days the relaxation following ricin treatment was at control values for rabbits given either dose of ricin. The relaxant effect of methacholine was significantly higher at 18 hours compared to later time periods ($p < 0.05$). An increased relaxation to methacholine indicates an increase in endothelial-dependent relaxation. An increased relaxation of the vasculature could contribute to the decreased blood pressure observed following ricin treatment.

2. To ATP

The responses of aorta rings contracted with norepinephrine to ATP are shown in Table 12. The relaxation to ATP was significantly reduced at 18 hours after a toxic sub-lethal dose of ricin ($p < 0.05$). The relaxations to ATP are also reduced at 4 days and 7 days following toxic sub-lethal dose ricin treatment and all time periods following minimum lethal dose ricin treatment, but were not significantly different from control ($p > 0.05$).

3. To Papaverine

The responses of aorta rings contracted with norepinephrine to papaverine are shown in Figures 51 to 56. There was no difference in the response of the aorta rings to papaverine in ricin treated rabbits compared to control.

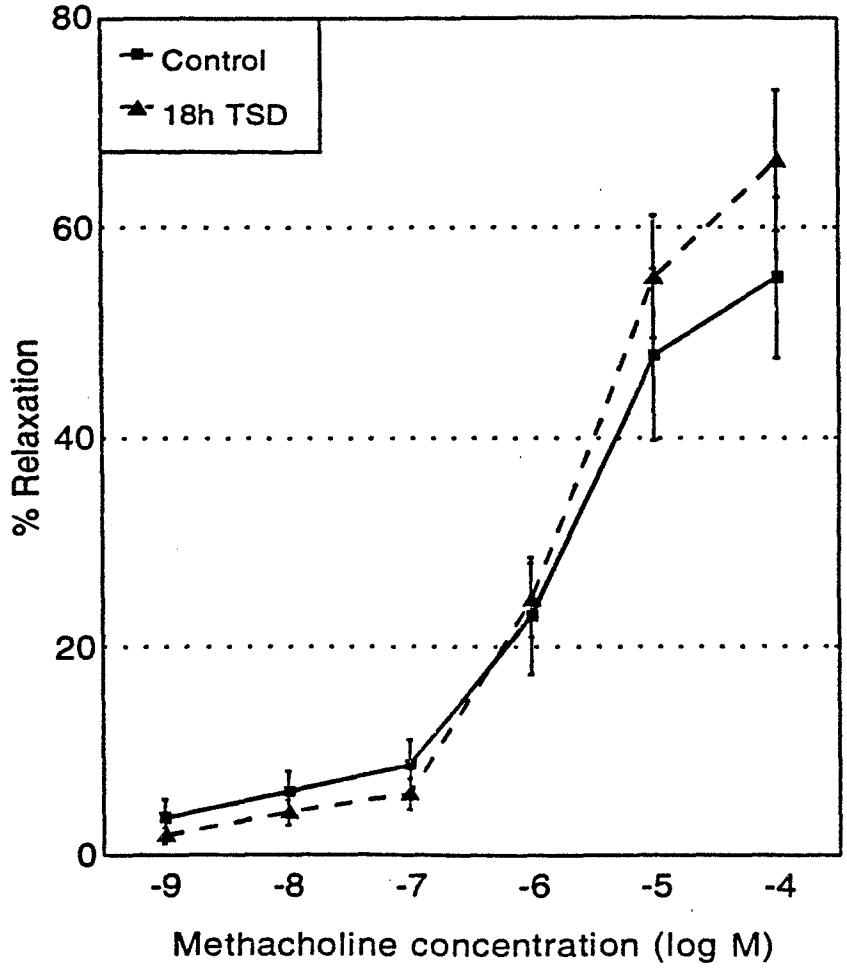

Figure 45. The effects of methacholine on aorta rings contracted with norepinephrine from rabbits receiving a toxic sub-lethal dose of ricin 18 hours earlier. Each point is the mean ± SEM from 11-22 rings from 6-11 rabbits.

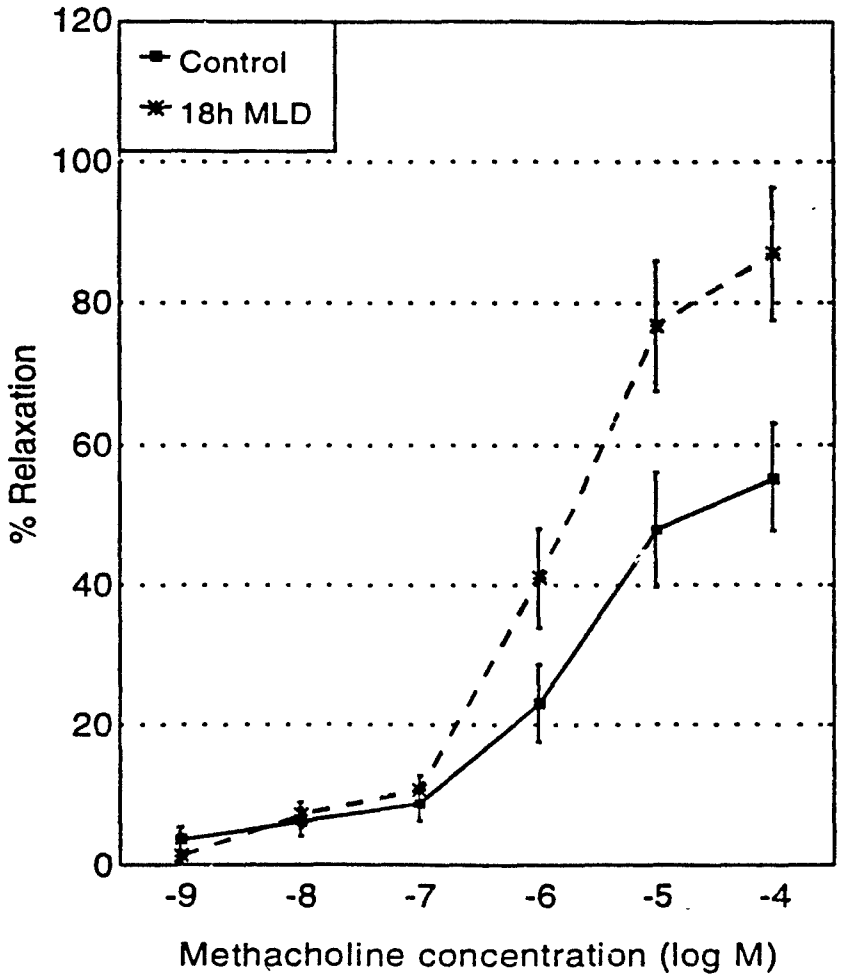

Figure 46. The effects of methacholine on aorta rings contracted with norepinephrine from rabbits receiving a minimum lethal dose of ricin 18 hours earlier. Each point is the mean ± SEM from 11-12 rings from 6-11 rabbits.

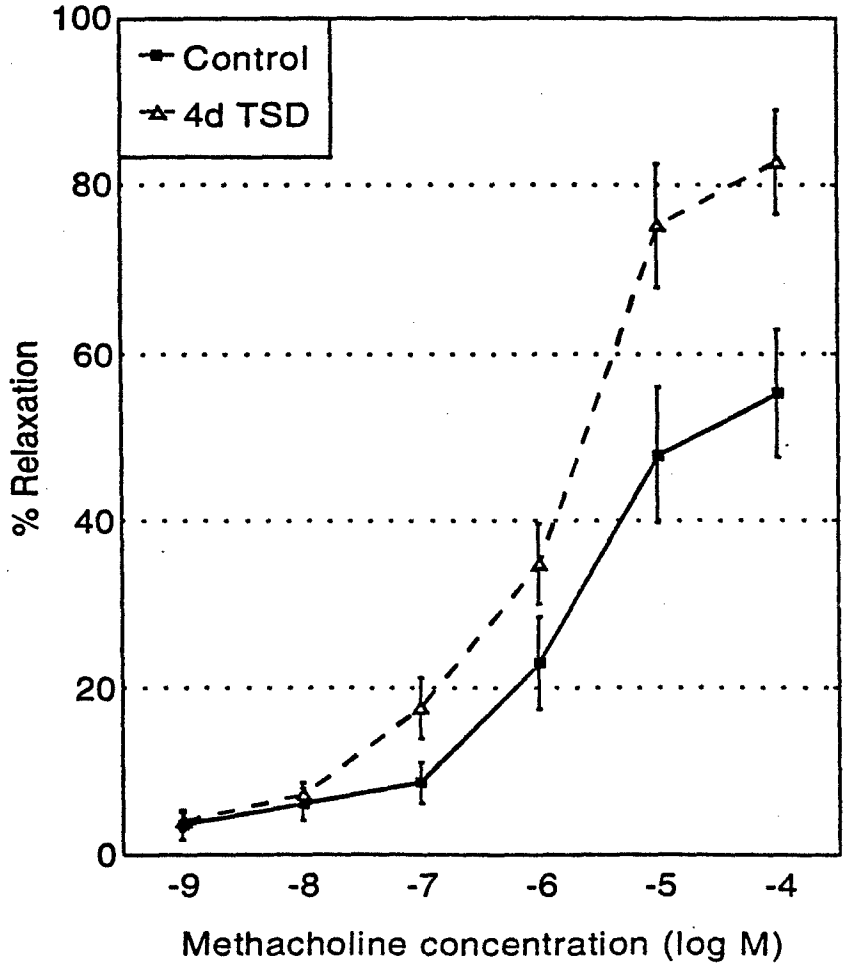

Figure 47. The effects of methacholine on aorta rings contracted with norepinephrine from rabbits receiving a toxic sub-lethal dose of ricin 4 days earlier. Each point is the mean ± SEM from 11-12 rings from 6 rabbits.

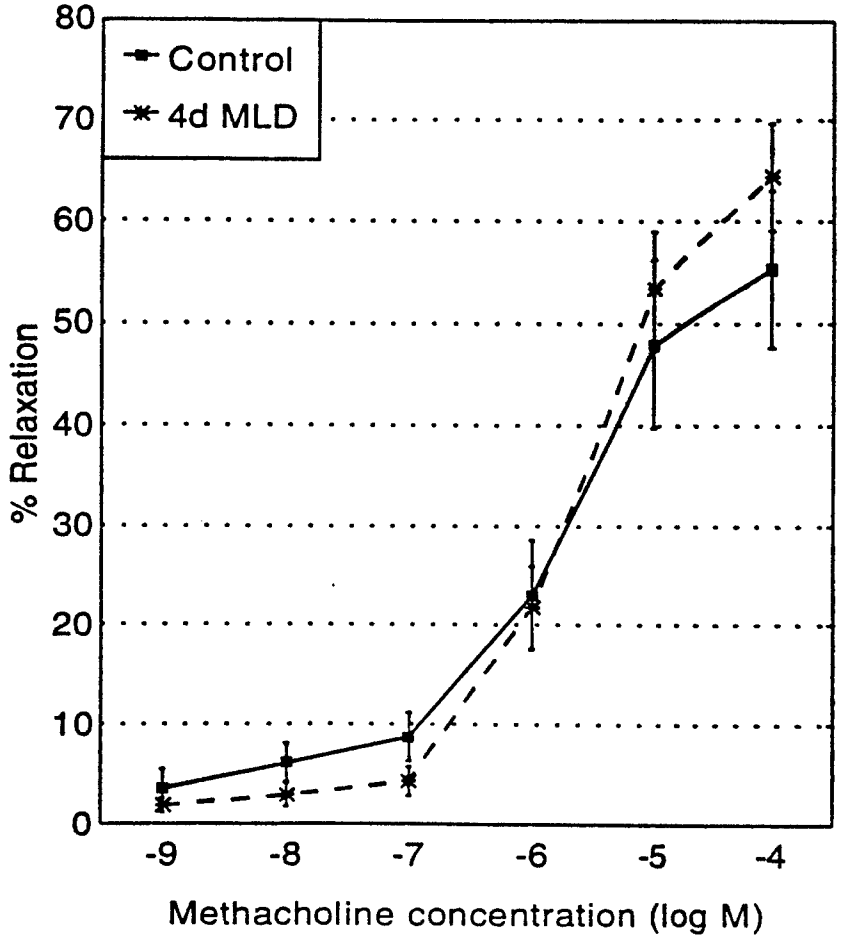

Figure 48. The effects of methacholine on aorta rings contracted with norepinephrine from rabbits receiving a minimum lethal dose of ricin 4 days earlier. Each point is the mean ± SEM from 11-15 rings from 6-7 rabbits.

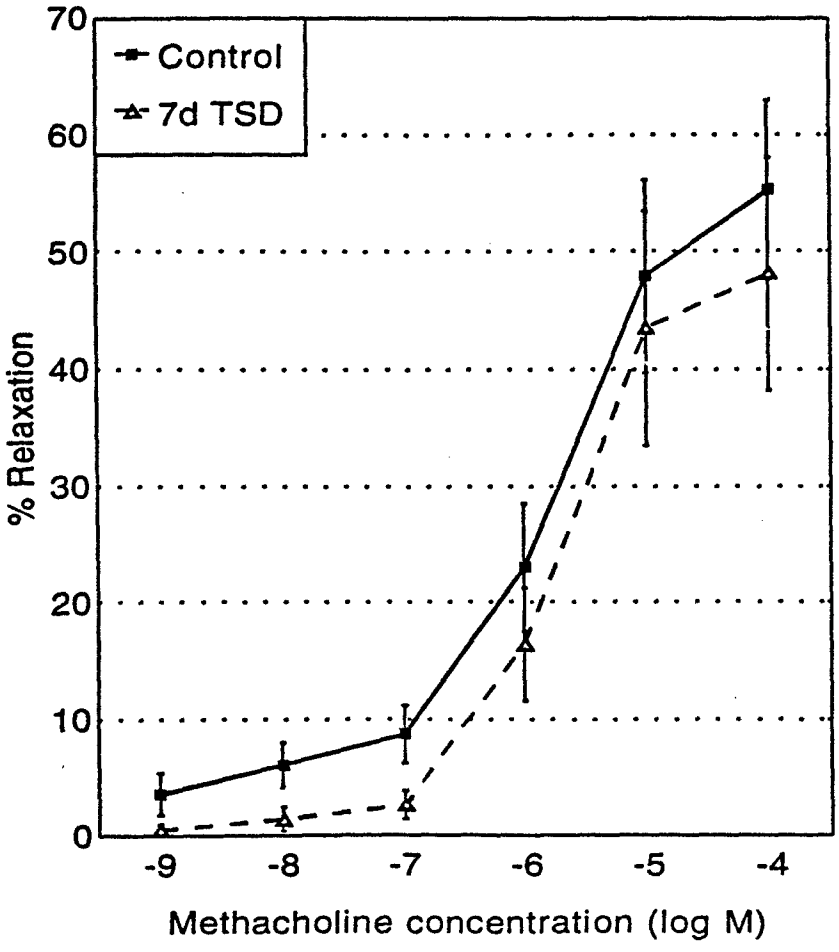

Figure 49. The effects of methacholine on aorta rings contracted with norepinephrine from rabbits receiving a toxic sub-lethal dose of ricin 7 days earlier. Each point is the mean ± SEM from 11-14 rings from 6-7 rabbits.

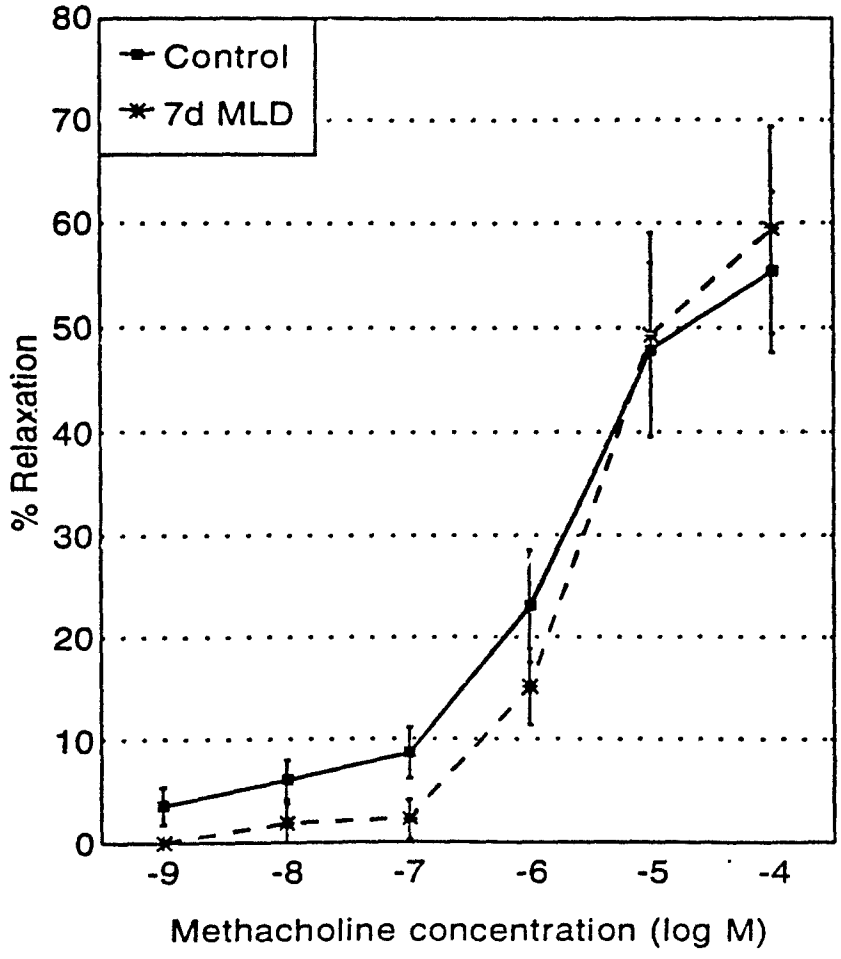

Figure 50. The effects of methacholine on aorta rings contracted with norepinephrine from rabbits receiving a minimum lethal dose of ricin 7 days earlier. Each point is the mean ± SEM from 11-12 rings from 6 rabbits.

Table 12. The Effects of ATP on Aorta Rings Contracted with Norepinephrine from Rabbits Given Ricin.

	Relaxation to 1μM ATP[a]			
		Time After Ricin Administration		
Treatment		**18 h**	**4 days**	**7 days**
Control	14.6±8.0			
Ricin, a toxic sub-lethal dose		3.1±0.7[b]	5.4±0.6	7.1±2.9
Ricin, a minimum lethal dose		11.3±2.0	7.4±1.7	7.1±2.9

a. Relaxations reported as % of initial norepinephrine contraction. Mean±SEM of 10-14 rings from 5-7 rabbits.

b. Significantly different from control $p<0.05$.

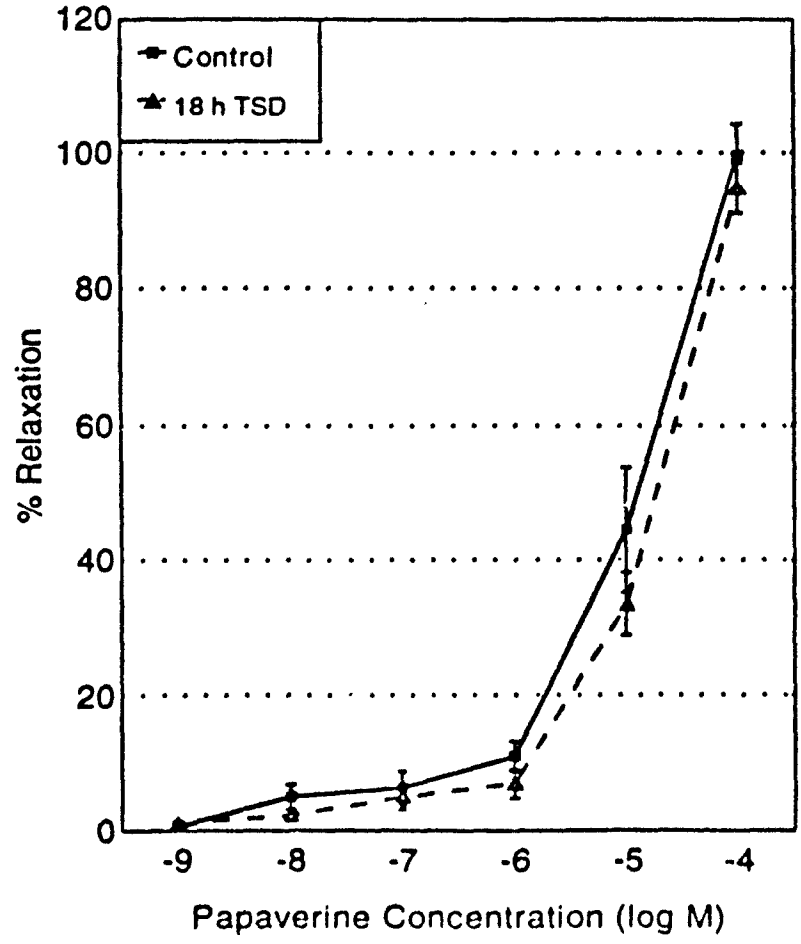

Figure 51. The effects of papaverine on aorta rings contracted with norepinephrine from rabbits receiving a toxic sub-lethal dose of ricin 18 hours earlier. Each point is the mean ± SEM of 11-22 rings from 6-11 rabbits.

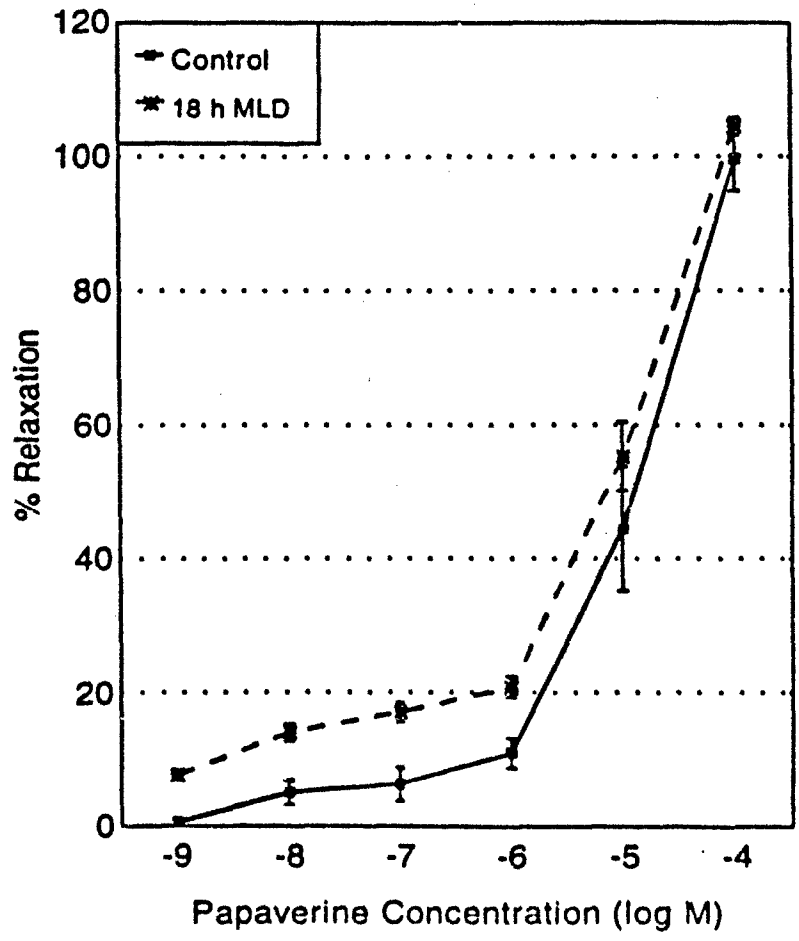

Figure 52. The effects of papaverine on aorta rings contracted with norepinephrine from rabbits receiving a minimum lethal dose of ricin 18 hours earlier. Each point is the mean ± SEM of 11-12 rings from 6 rabbits.

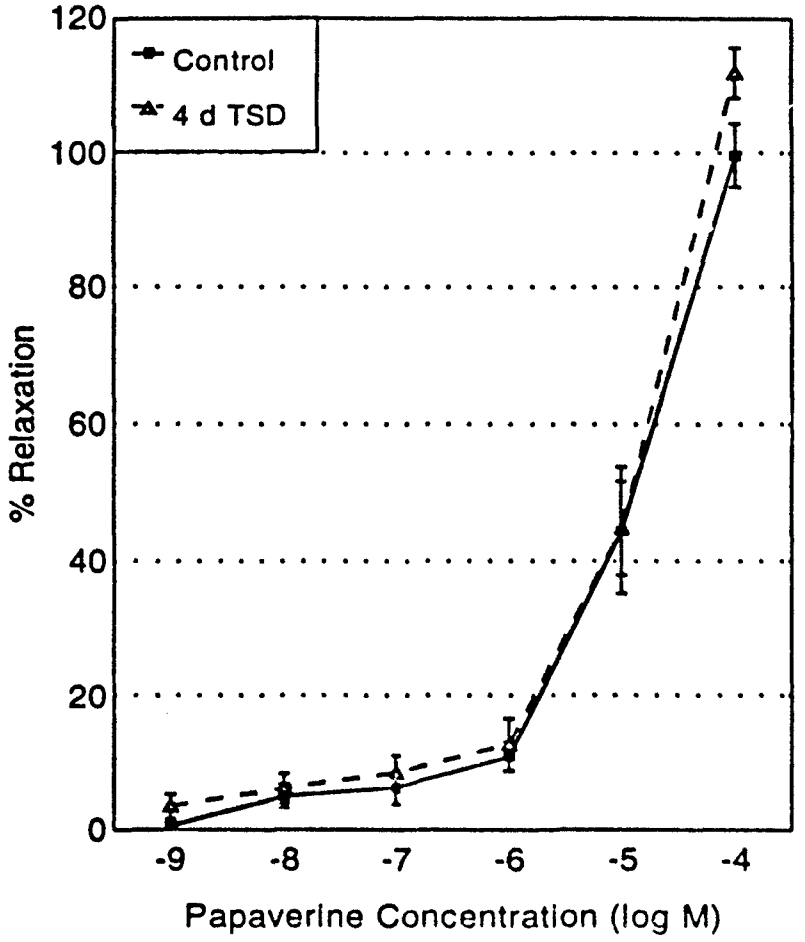

Figure 53. The effects of papaverine on aorta rings contracted with norepinephrine from rabbits receiving a toxic sub-lethal dose of ricin 4 days earlier. Each point is the mean ± SEM of 11-12 rings from 6 rabbits.

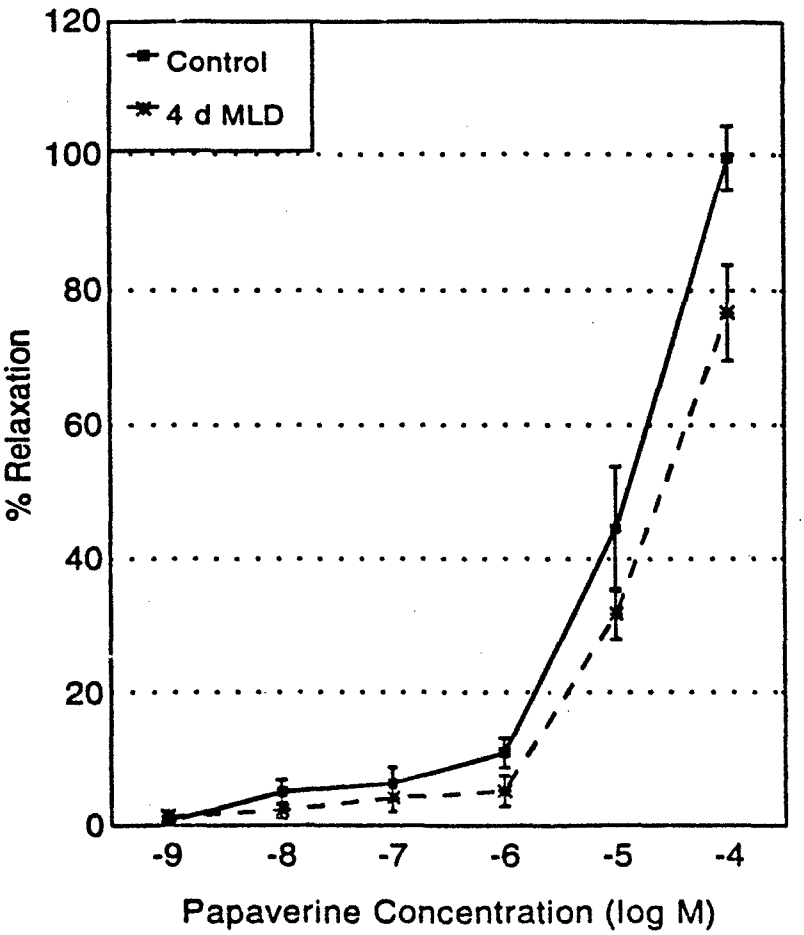

Figure 54. The effects of papaverine on aorta rings contracted with norepinephrine from rabbits receiving a minimum lethal dose of ricin 4 days earlier. Each point is the mean ± SEM of 11-15 rings from 6-7 rabbits.

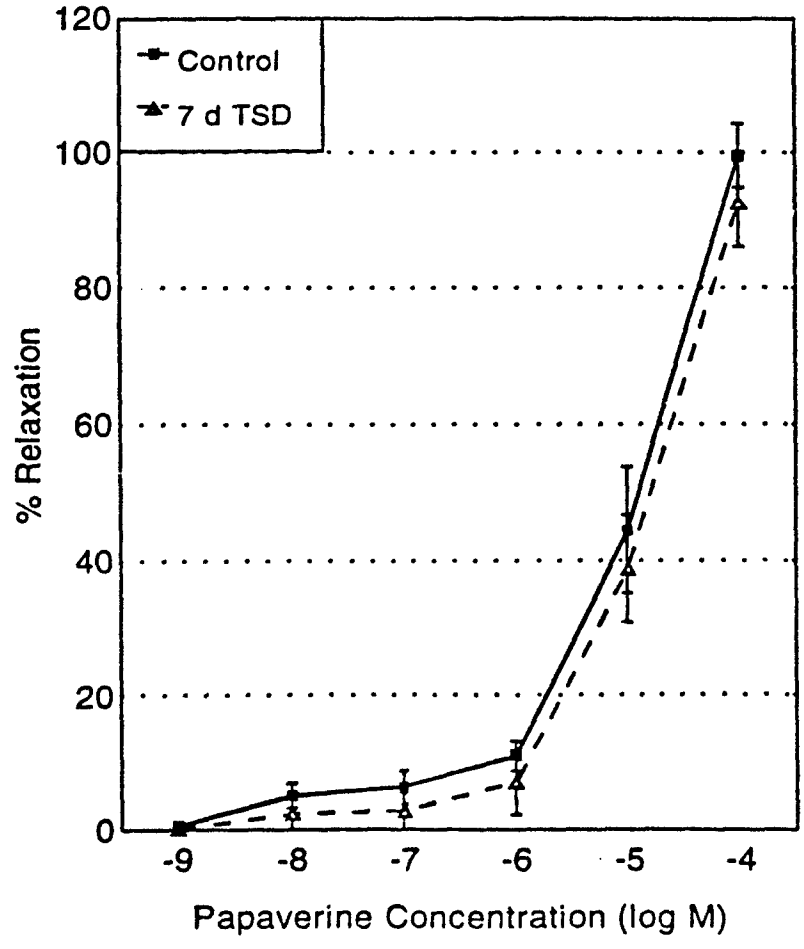

Figure 55. The effects of papaverine on aorta rings contracted with norepinephrine from rabbits receiving a toxic sub-lethal dose of ricin 7 days earlier. Each point is the mean ± SEM of 11-14 rings from 6-7 rabbits.

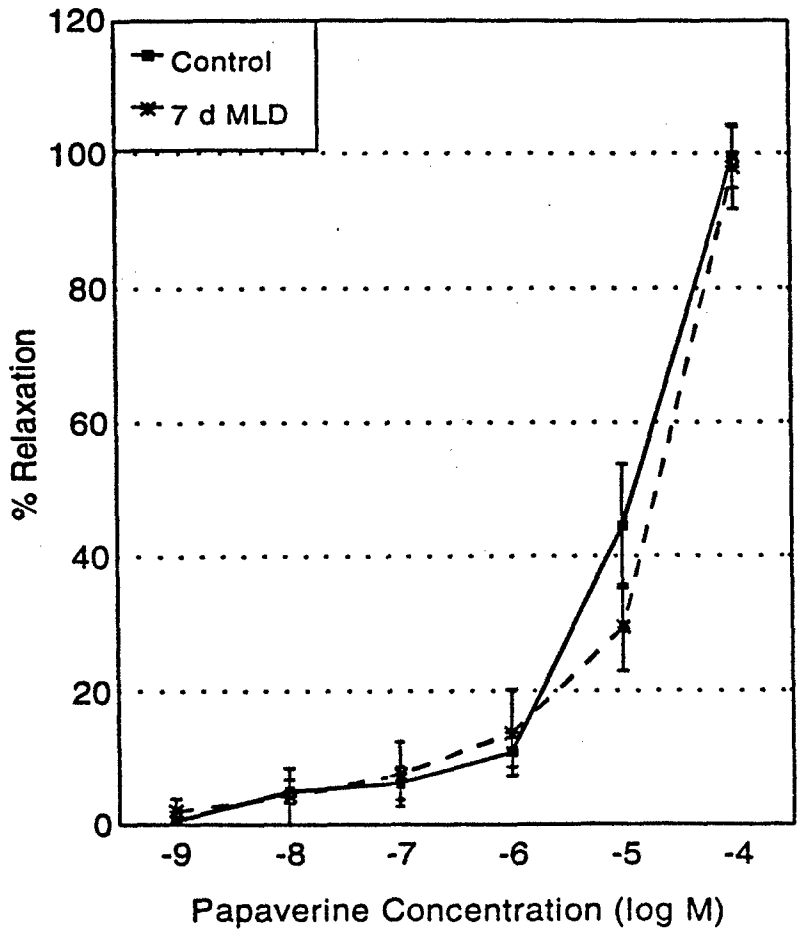

Figure 56. The effects of papaverine on aorta rings contracted with norepinephrine from rabbits receiving a minimum lethal dose of ricin 7 days earlier. Each point is the mean ± SEM of 11-12 rings from 6 rabbits.

I. Effects of Ricin Administration to Rabbits on the Norepinephrine Content of Their Thoracic Aortas and Plasma.

The plasma of control as well as treated rabbits contained detectable quantities of norepinephrine (Table 13). There was no significant difference between the norepinephrine content of plasma from ricin treated animals compared to that of the plasma from control rabbits.

The aorta of control as well as treated rabbits contained detectable quantities of norepinephrine (Table 14). All of the treatment groups had a higher level of norepinephrine measured in the aorta, but only the rabbits in the 4 day toxic sub-lethal dose group had a significantly elevated norepinephrine concentration ($p < 0.05$).

J. Effects of Ricin Administration to Rabbits on Norepinephrine Released from the Aorta During Transmural Nerve Stimulation.

Norepinephrine was released by the nerves in the rabbit aorta as evidenced by an increased washout of radioactively labeled norepinephrine during periods of electrical stimulation (Figures 57 to 62). The fraction of norepinephrine efflux to a 2 Hz transmural stimulation was not altered by treatment of the rabbit with either a minimum lethal dose or a toxic sub-lethal dose of ricin (Table 15). However, the fractional efflux to a 10 Hz stimulation was increased in the group of rabbits treated with a toxic sub-lethal dose of ricin and euthanized 18 hr post injection. This increase was significant for both 10 Hz stimulations ($p < 0.05$). The increase could indicate an increase in norepinephrine released by transmural stimulation or a decrease in activity of the amine pump. Either or both would result in an increased washout of norepinephrine. Although none of the other treatment groups showed any significant difference in either the 2 Hz or 10 Hz stimulations as compared to control, the 4 day Toxic Sub-lethal Dose group also was increased above control values at both 10 Hz stimulations. The efflux for the rabbits in the 18 hour and 7 day minimum lethal dose groups were also increased compared to those in the control group.

When the fraction of norepinephrine efflux was converted to the nanograms of norepinephrine efflux/mg of tissue (Table 16), the first 2 Hz stimulation as well as both 10 Hz stimulations in the 18 hr toxic sub-lethal dose group were significantly increased as compared to control ($p < 0.05$).

Table 13. Norepinephrine Content[a] of Plasma From Rabbits Given Ricin I.V.

		Time Period From Ricin Administration to Obtaining Plasma.		
	Control Mean±SEM	18 hours Mean±SEM	4 days Mean±SEM	7 days Mean±SEM
Ricin, at toxic sub-lethal dose	5.10±0.55	4.46±0.30	5.35±0.31	6.43±1.11
Ricin, at minimum lethal dose	5.10±0.55	4.66±0.53	5.35±0.40	4.56±0.21

a. ng norepinephrine/ml of plasma. Each value is the mean±SEM from 6 samples from six different rabbits.

Table 14. Norepinephrine Content[a] of Thoracic Aortas From Rabbits Given Ricin I.V.

		Time Period From Ricin Administration to Removing Aorta.		
	Control Mean±SEM	18 hours Mean±SEM	4 days Mean±SEM	7 days Mean±SEM
Ricin, a toxic sub-lethal dose	2.06±0.26	2.83±0.42	3.28[b]±0.34	2.81±0.20
Ricin, a minimum lethal dose	2.06±0.26	2.08±0.16	2.64±0.44	2.40±0.25

a. ng norepinephrine/mg wet weight of tissue. Each value is the mean±SEM from 6 aortas from six different rabbits.

b. Different from control ($p<0.05$), by Dunnett's test.

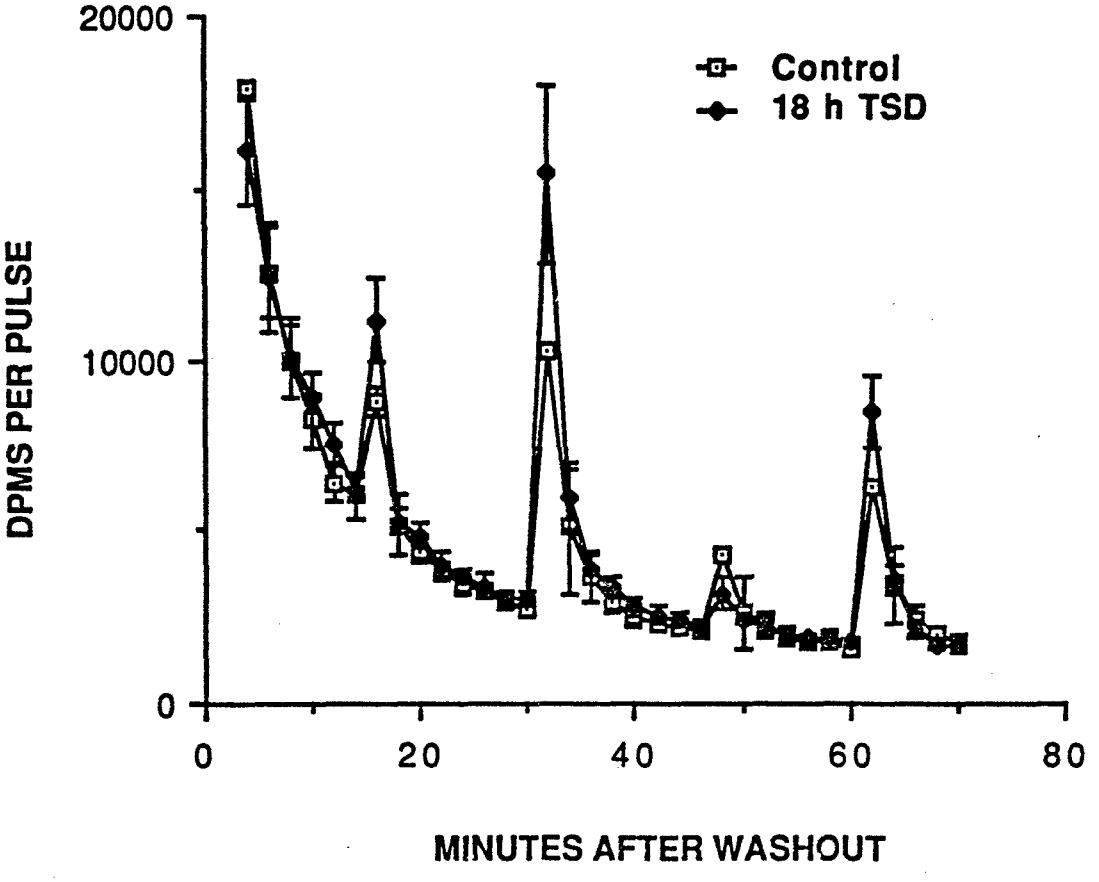

Figure 57. Washout of radioactivity from the helically-cut aorta following incubation of the tissue with tritiated NE. Strips were stimulated transmurally for 90 sec at 14 and 44 min at 2 Hz, and at 28 and 60 min at 10 Hz. Aortas were from rabbits given a toxic sub-lethal dose of ricin 18 hours earlier. Each point is the mean ± SEM from 12 strips from 6 animals.

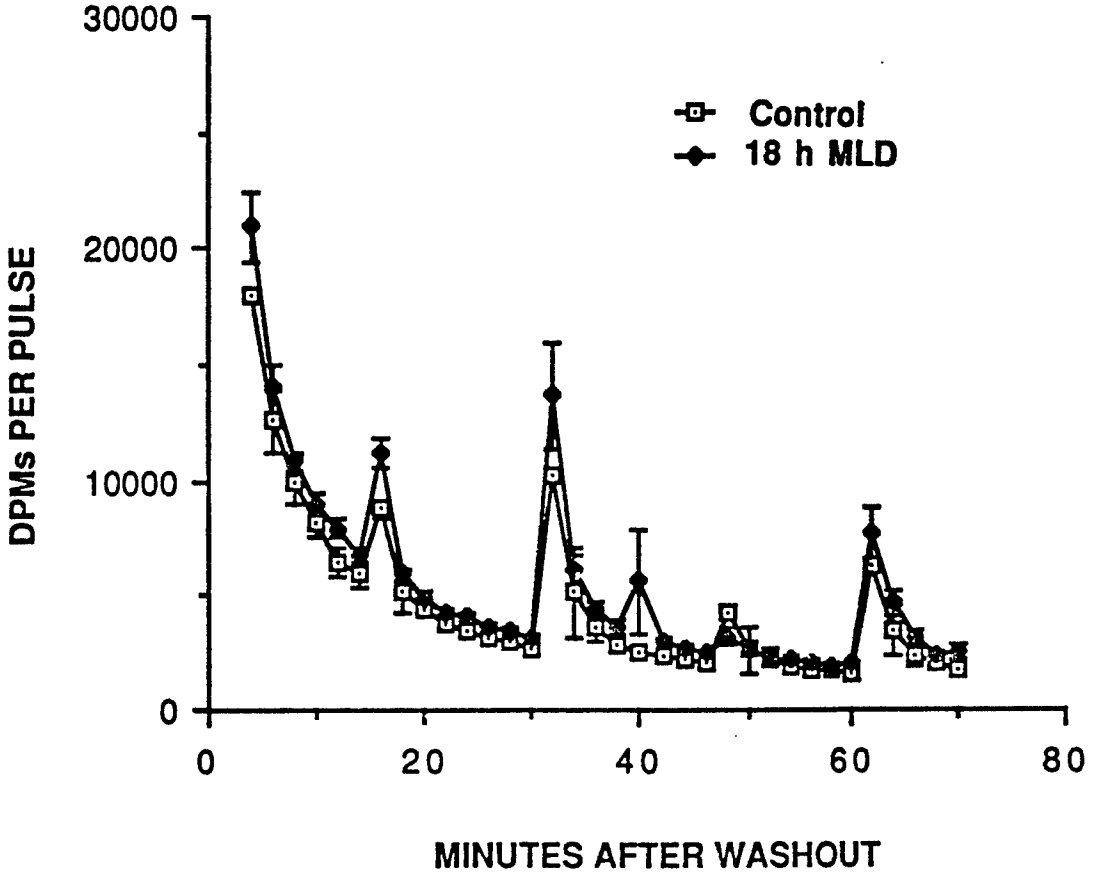

Figure 58. Washout of radioactivity from the helically-cut aorta following incubation of the tissue with tritiated NE. Strips were stimulated transmurally for 90 sec at 14 and 44 min at 2 Hz, and at 28 and 60 min at 10 Hz. Aortas were from rabbits given a minimum lethal dose of ricin 18 hours earlier. Each point is the mean ± SEM from 12 strips from 6 animals.

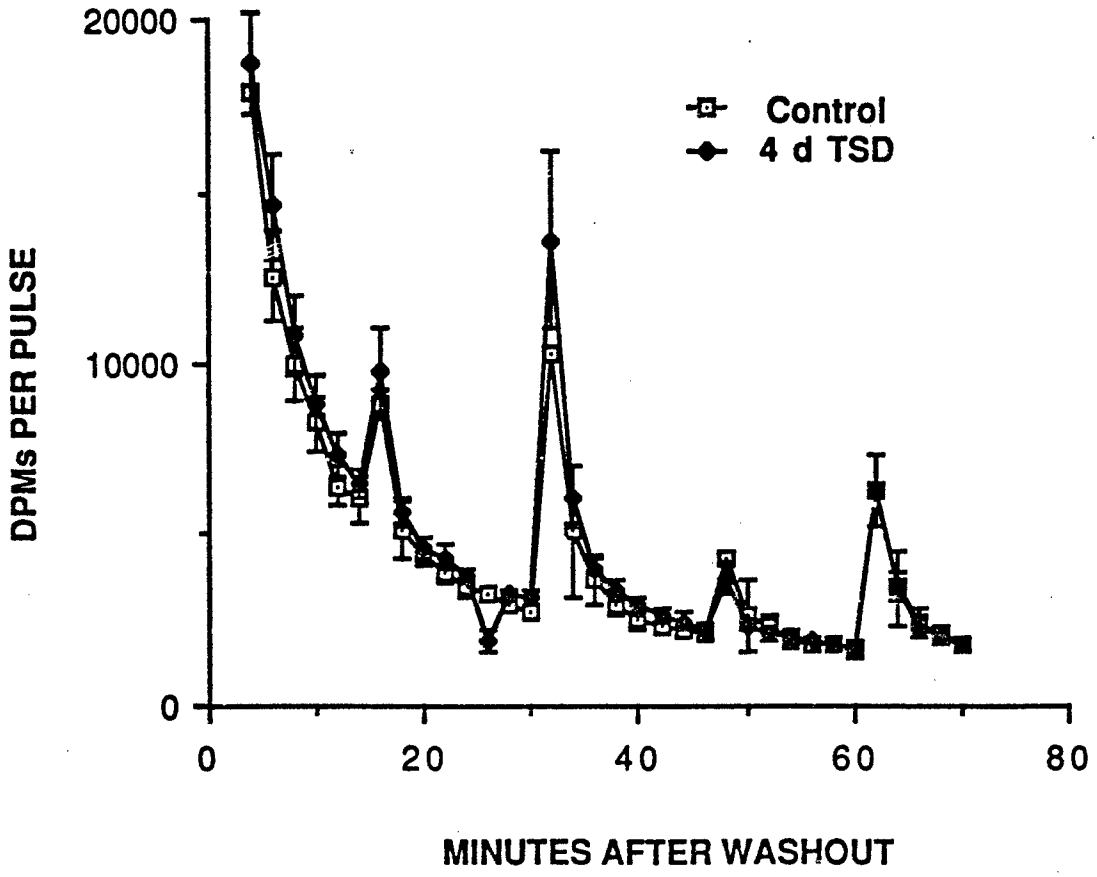

Figure 59. Washout of radioactivity from the helically-cut aorta following incubation of the tissue with tritiated NE. Strips were stimulated transmurally for 90 sec at 14 and 44 min at 2 Hz, and at 28 and 60 min at 10 Hz. Aortas were from rabbits given a toxic sub-lethal dose of ricin 4 days earlier. Each point is the mean ± SEM from 12 strips from 6 animals.

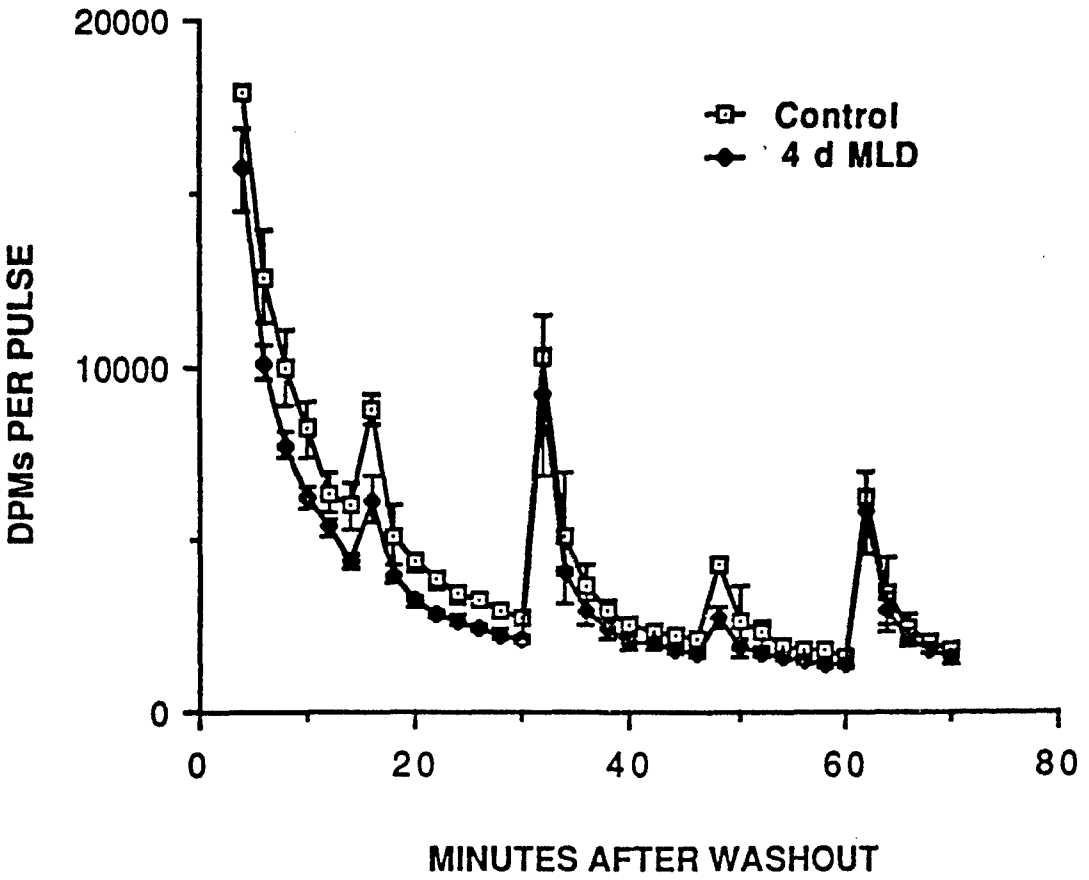

Figure 60. Washout of radioactivity from the helically-cut aorta following incubation of the tissue with tritiated NE. Strips were stimulated transmurally for 90 sec at 14 and 44 min at 2 Hz, and at 28 and 60 min at 10 Hz. Aortas were from rabbits given a minimum lethal dose of ricin 4 days earlier. Each point is the mean ± SEM from 9-12 strips from 5-6 animals.

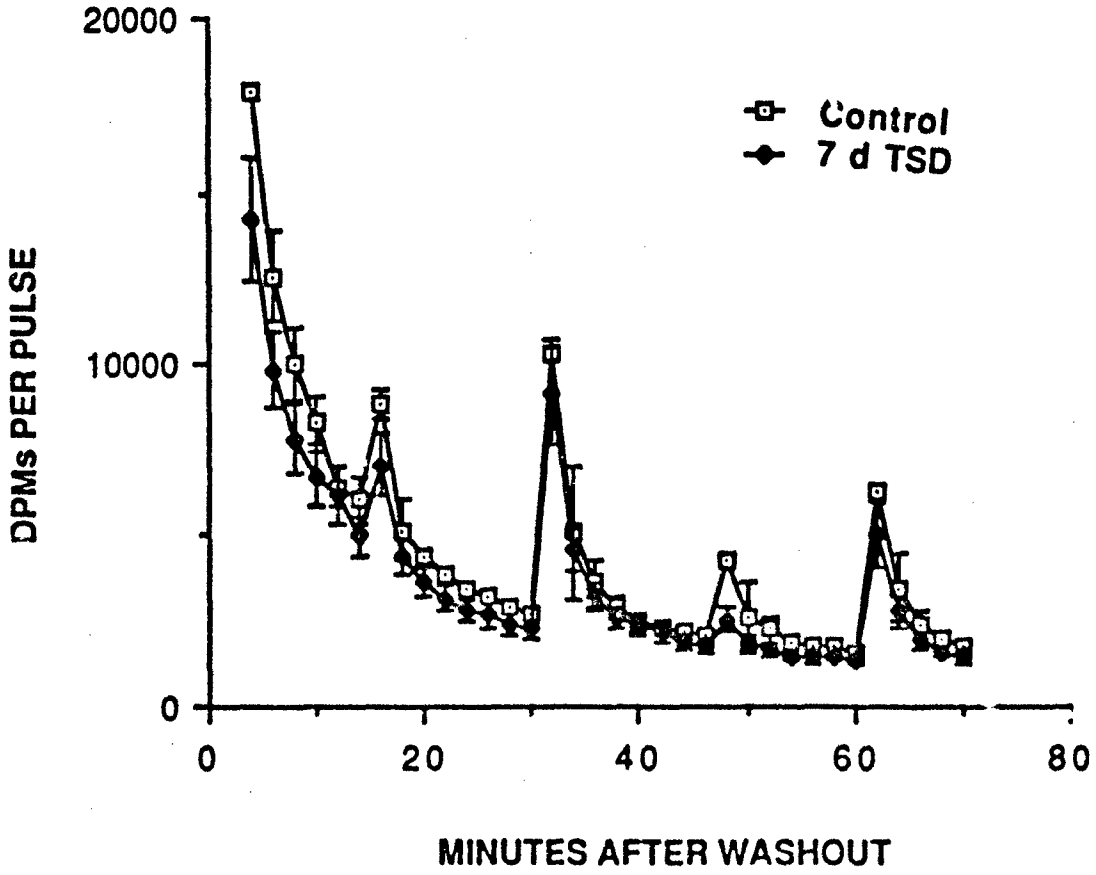

Figure 61. Washout of radioactivity from the helically-cut aorta following incubation of the tissue with tritiated NE. Strips were stimulated transmurally for 90 sec at 14 and 44 min at 2 Hz, and at 28 and 60 min at 10 Hz. Aortas were from rabbits given a toxic sub-lethal dose of ricin 7 days earlier. Each point is the mean ± SEM from 12 strips from 6 animals.

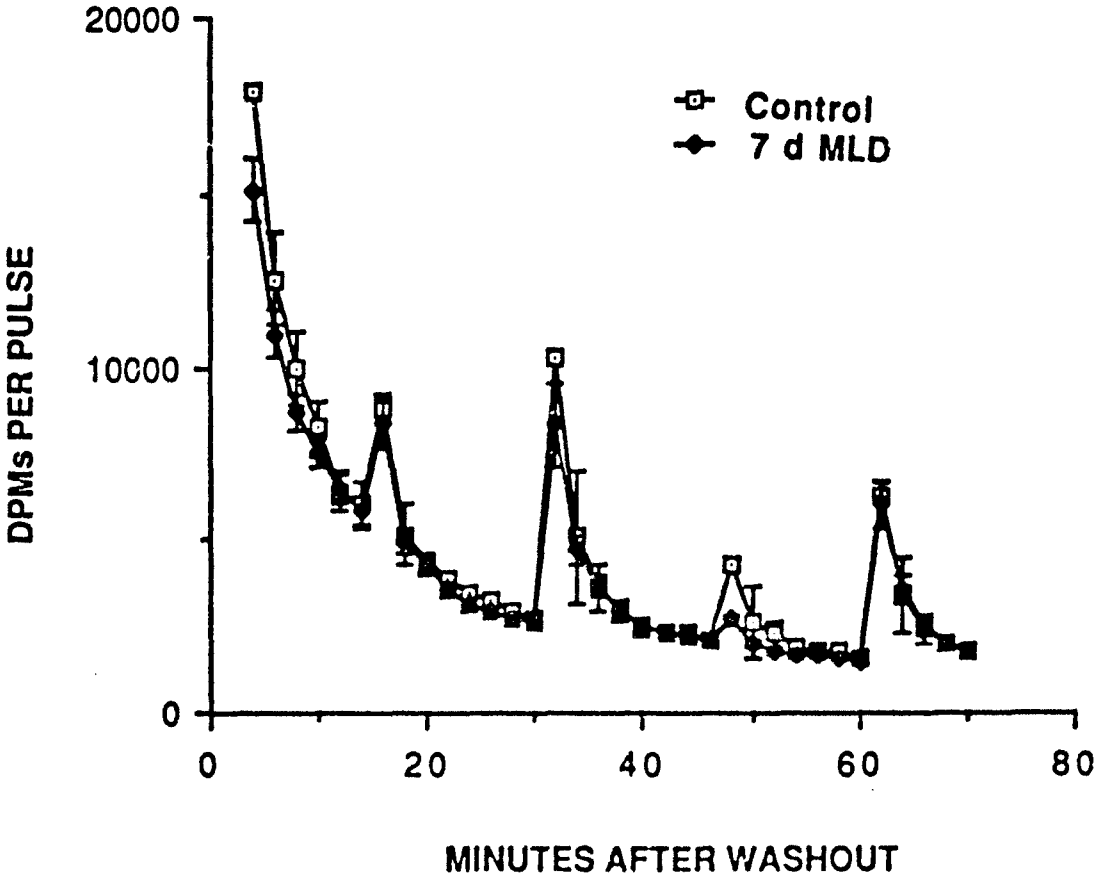

Figure 62. Washout of radioactivity from the helically-cut aorta following incubation of the tissue with tritiated NE. Strips were stimulated transmurally for 90 sec at 14 and 44 min at 2 Hz, and at 28 and 60 min at 10 Hz. Aortas were from rabbits given a minimum lethal dose of ricin 7 days earlier. Each point is the mean ± SEM from 12 strips from 6 animals.

Table 15. The Fraction of Norepinephrine Efflux from Rabbit Aorta by Each Pulse During Transmural Stimulation at Intervals Following Ricin Administration.

	Norepinephrine Release (as fraction x 10^{-4} of NE in the aorta)			
	1st 2 Hz	2nd 2 Hz	1st 10 Hz	2nd 10 Hz
Treatment	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
Control	1.01±0.22	1.81±1.05	0.86±0.20	0.88±0.24
Ricin, a toxic sub-lethal dose				
18 hrs	1.87±0.22	1.06±0.26	1.98±0.31*	1.84±0.26*
4 days	1.23±0.20	0.93±0.21	1.09±0.21	1.02±0.17
7 days	0.88±0.18	0.58±0.11	0.87±0.15	0.75±0.15
Ricin, a minimum lethal dose				
18 hrs	1.39±0.14	0.67±0.16	0.98±0.17	1.41±0.27
4 days	0.88±0.32	0.71±0.20	0.87±0.22	0.88±0.22
7 days	1.10±0.33	0.54±0.09	0.96±0.15	1.22±0.12

n = 9-12 strips from 5-6 animals.

* Significantly different from control ($p < 0.05$).

Table 16. Efflux of Norepinephrine from Rabbit Aorta During Transmural Stimulation at Intervals Following Ricin Administration.

	Norepinephrine Release[a] (ng mg tissue $^{-1}$ pulse $^{-1}$)			
	1st 2 Hz	2nd 2 Hz	1st 10 Hz	2nd 10 Hz
Treatment	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
Control	0.21±0.05	0.40±0.21	0.18±0.04	0.18±0.05
Ricin, a toxic sub-lethal dose				
18 hrs	0.48±0.08*	0.32±0.07	0.56±0.09*	0.52±0.08*
4 days	0.41±0.06	0.31±0.07	0.36±0.07	0.33±0.05
7 days	0.25±0.05	0.17±0.03	0.23±0.05	0.22±0.04
Ricin, a minimum lethal dose				
18 hrs	0.27±0.04	0.13±0.03	0.24±0.03	0.29±0.06
4 days	0.23±0.09	0.19±0.05	0.23±0.06	0.23±0.06
7 days	0.26±0.08	0.13±0.02	0.22±0.03	0.30±0.03

a. Calculated from fractional efflux and norepinephrine content.

Incubated for 90 min. in labeled 0.1 μM norepinephrine.

n = 9-12 strips from 5-6 animals.

* Significantly different from control ($p < 0.05$).

K. Effects of Ricin Administration to Rabbits on Norepinephrine Uptake by the Aorta.

The aortas of rabbits in both the control and ricin treated groups did take up norepinephrine as shown in Table 17. There was no significant difference in the uptake of norepinephrine in any of the treated groups as compared to control.

L. Effects of Ricin Administration to Rabbits on Monoamine Oxidase Activity of Various Tissues.

Monoamine oxidase activity was determined in several tissues for rabbits given a toxic sub-lethal dose of ricin (Table 18) and a minimum lethal dose of ricin (Table 19). No significant difference was detected in any tissue for any of the rabbits given ricin compared to control. In the heart it was noted that MAO activity was decreased at all time periods following ricin treatment at both dose levels even though none were statistically significant ($p > 0.05$). A decrease in MAO activity in the heart could increase norepinephrine concentrations which could predispose the heart to arrhythmias.

M. Effects of Ricin Administration to Rabbits on Catechol-*O*-Methyltransferase Activity of the Aorta.

Catechol-*O*-methyl transferase activity was detectable in the thoracic aorta of control and ricin-treated rabbits (Table 20). No significant difference ($p > 0.05$) was found in activity between control and ricin treated rabbits.

N. Effects of Ricin Administration to Rabbits on Cyclic-AMP Activity in the Plasma.

All plasma samples had detectable cAMP concentrations (Table 21). These concentrations were decreased by over 70 percent ($p < 0.05$) at 18 hours, but had partially recovered at four days, with cAMP concentrations then not different ($p > 0.05$) from control.

O. Effects of Ricin Administration to Rabbits on Calcium Uptake into the Rabbit Aorta.

The effects of ricin administration to rabbits on the time course of basal ^{45}Ca uptake in isolated aortas are shown in Figures 63-68. There were no differences in basal ^{45}Ca uptake between control and ricin-treated rabbits ($p > 0.05$).

The 100 μM NE-stimulated ^{45}Ca uptake (Tables 22 and 23) in ricin-treated rabbits was less than control at 18h at both ricin doses although not significantly different from control ($p > 0.05$). Calcium uptake had partially recovered by 4 and 7 days following both ricin doses. The 80 mM KCl-induced ^{45}Ca uptake was significantly depressed ($p < 0.05$) in aortas from rabbits given both ricin doses and sacrificed at 18h (Tables 22 and 23). By 4 and 7 days calcium uptake capability had partially or fully recovered following both ricin doses.

P. Effects of Ricin Administration to Rabbits on Calcium Efflux From the Rabbit Aorta.

The ^{45}Ca efflux rates (expressed as fraction of ^{45}Ca lost/min) from aortas from control and ricin-treated rabbits are shown in Figures 69-74. There were no differences in basal ^{45}Ca efflux rate for the first five 5-min intervals among control and ricin-treated rabbits. The 80 mM KCl-induced ^{45}Ca efflux rates (Table 24) were significantly increased ($p < 0.05$) only in aortas from rabbits receiving a minimum lethal dose of ricin and sacrificed at 7d, while the 100 μM NE-induced ^{45}Ca efflux rate (Table 25) was significantly increased ($p < 0.05$) in aortas from rabbits receiving the minimum lethal dose of ricin and sacrificed at 4 or 7d.

Table 17. Norepinephrine Uptake by Rabbit Aorta Following Ricin Administration[a].

RICIN DOSE	TIME PERIOD FROM RICIN ADMINISTRATION TO OBTAINING TISSUES			
		18h	4d	7d
	Mean±S.E.M. (n)	Mean±S.E.M. (n)	Mean±S.E.M. (n)	Mean±S.E.M. (n)
Control	0.683±0.10 (6)			
Toxic Sub-lethal Dose		0.541±0.07 (6)	0.710±0.10 (6)	0.571±0.06 (6)
Minimum Lethal Dose		0.580±0.05 (6)	0.741±0.11 (5)	0.593±0.04 (6)

[a] Expressed as pM/mg wet weight tissue.

Table 18. Monoamine Oxidase Activity[a] of Various Tissues in Rabbits Given a Toxic Sublethal Dose of Ricin i.v.

TISSUE	CONTROL	Time Period After Ricin Administration To Obtaining Tissues		
		18 HOURS	4 DAYS	7 DAYS
	Mean±SEM(n)	Mean±SEM(n)	Mean±SEM(n)	Mean±SEM(n)
Thoracic Aorta (upper 2/3)	5.1±0.9 (7)	6.2±1.3 (6)	8.4±2.1 (6)	6.1±1.0 (7)
Brain Stem	1.6±0.6 (7)	1.7±0.6 (6)	3.3±1.8 (6)	1.7±0.8 (7)
Cerebellum	1.8±0.6 (7)	1.5±0.4 (6)	2.4±1.2 (6)	1.6±0.2 (7)
Cerebrum	2.6±0.8 (7)	4.1±1.6 (6)	1.5±0.3 (6)	2.0±0.3 (7)
Heart	5.1±1.9 (7)	2.5±0.4 (6)	3.7±1.8 (6)	3.2±0.7 (7)
Liver	40.4±6.7 (7)	34.9±5.1 (6)	21.0±2.3 (6)	31.2±5.6 (7)
Lung	13.0±2.0 (7)	9.4±0.5 (6)	10.4±0.6 (6)	9.6±0.9 (7)
Thoracic Aorta (lower 1/3)	4.3±1.7 (7)	6.6±1.5 (6)	9.6±4.1 (6)	4.5±0.9 (7)

a = activity is expressed as n moles substrate deaminated per gram wet weight of tissue per minute.

Table 19. Monoamine Oxidase Activity[a] of Various Tissues in Rabbits Given a Minimum Lethal Dose of Ricin i.v.

TISSUE	CONTROL	Time Period After Ricin Administration to Obtaining Tissues		
		18 HOURS	4 DAYS	7 DAYS
	Mean±SEM(n)	Mean±SEM(n)	Mean±SEM(n)	Mean±SEM(n)
Thoracic Aorta (upper 2/3)	5.1±0.9 (7)	4.7±0.7 (6)	6.6±0.8 (6)	4.8±0.6 (7)
Brain Stem	1.6±0.6 (7)	2.5±0.6 (6)	2.7±0.8 (6)	3.6±1.2 (7)
Cerebellum	1.8±0.6 (7)	2.7±0.4 (6)	4.7±2.9 (6)	2.6±0.6 (7)
Cerebrum	2.6±0.8 (7)	3.7±0.8 (6)	2.4±0.5 (6)	2.6±0.5 (7)
Heart	5.1±1.9 (7)	2.4±0.2 (6)	4.1±1.1 (6)	2.0±0.4 (7)
Liver	40.4±6.7 (7)	42.9±4.1 (6)	37.0±5.2 (6)	42.9±9.7 (7)
Lung	13.0±2.0 (7)	13.1±1.2 (6)	18.0±3.5 (6)	10.5±1.8 (7)
Thoracic Aorta (lower 1/3)	4.3±1.7 (7)	4.0±0.3 (6)	7.5±1.0 (6)	4.8±0.6 (7)

a = activity is expressed as n moles substrate deaminated per gram wet weight of tissue per minute.

Table 20. Catechol-*O*-Methyl Transferase Activity in Rabbit Aorta Following Ricin Administration.[1]

RICIN DOSE	TIME PERIOD FROM RICIN ADMINISTRATION TO OBTAINING TISSUES			
		18h	4d	7d
	Mean±S.E.M. (n)	Mean±S.E.M. (n)	Mean±S.E.M. (n)	Mean±S.E.M. (n)
Control	0.390±0.05 (7)			
Toxic Sub-lethal Dose		0.483±0.14 (5)	0.544±0.10 (6)	0.348±0.06 (6)
Minimum Lethal Dose		0.281±0.09 (4)	0.421±0.03 (6)	0.253±0.03 (6)

[1]Expressed as nM product formed/g wet weight tissue/min.

Table 21. Plasma Cyclic AMP Activity (pmol/ml) Following Administration of a Minimum Lethal Dose of Ricin i.v. to Rabbits.

	TIME PERIOD FROM RICIN ADMINISTRATION TO OBTAINING TISSUES	
CONTROL	18 hours	4 days
Mean±S.E.M. (n)	Mean±S.E.M. (n)	Mean±S.E.M. (n)
54.8±13.4 (6)	15.6±2.5* (5)	33.2±3.4 (3)

*Different from control, $p < 0.05$, Duncan's New Multiple Range Test.

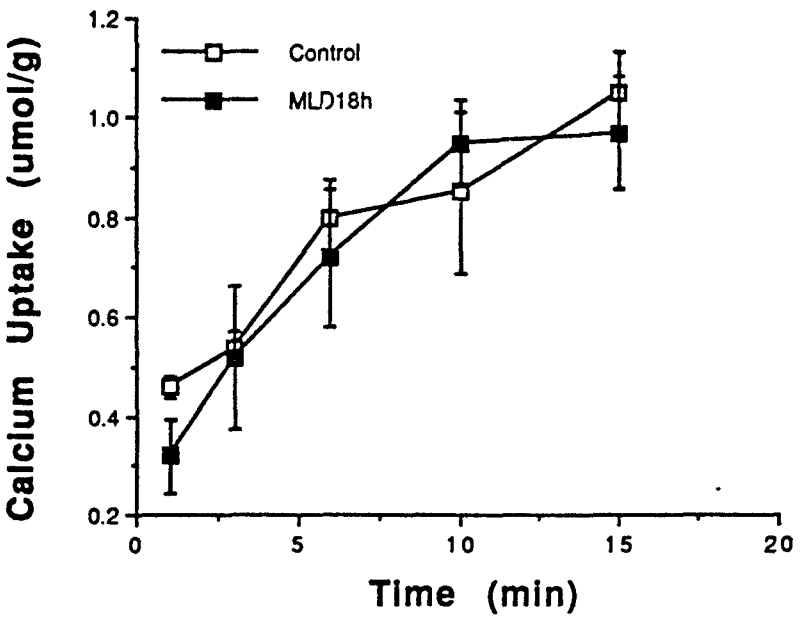

Fig. 63. The effects of ricin administration to rabbits on basal ^{45}Ca uptake in isolated aortas 18h after i.v. injection of a minimum lethal dose of ricin. Each point is the mean ± S.E.M. of aorta pieces from 4 rabbits.

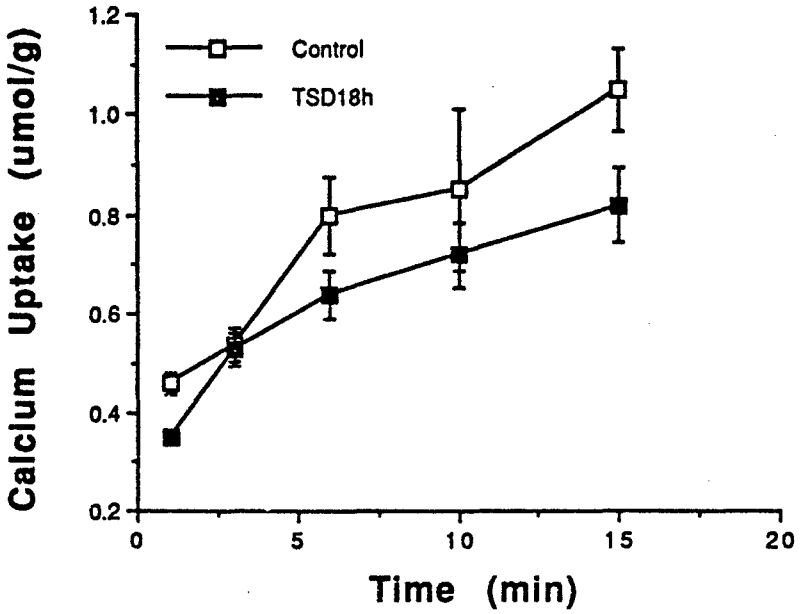

Fig. 64. The effects of ricin administration to rabbits on basal ^{45}Ca uptake in isolated aortas 18h after i.v. injection of a toxic sublethal dose of ricin. Each point is the mean ± S.E.M. of aorta pieces from 4 rabbits.

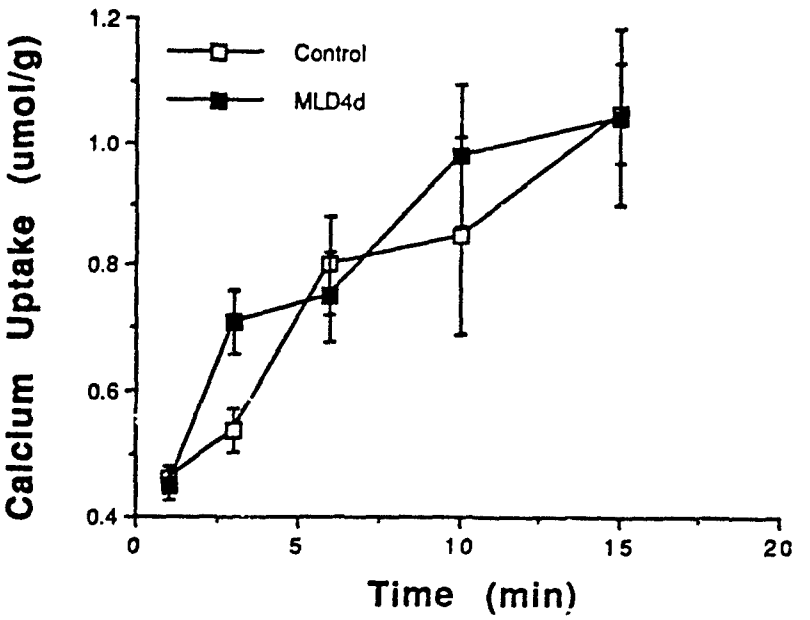

Fig. 65. The effects of ricin administration to rabbits on basal ^{45}Ca uptake in isolated aortas 4d after i.v. injection of a minimum lethal dose of ricin. Each point is the mean ± S.E.M. of aorta pieces from 4-6 rabbits.

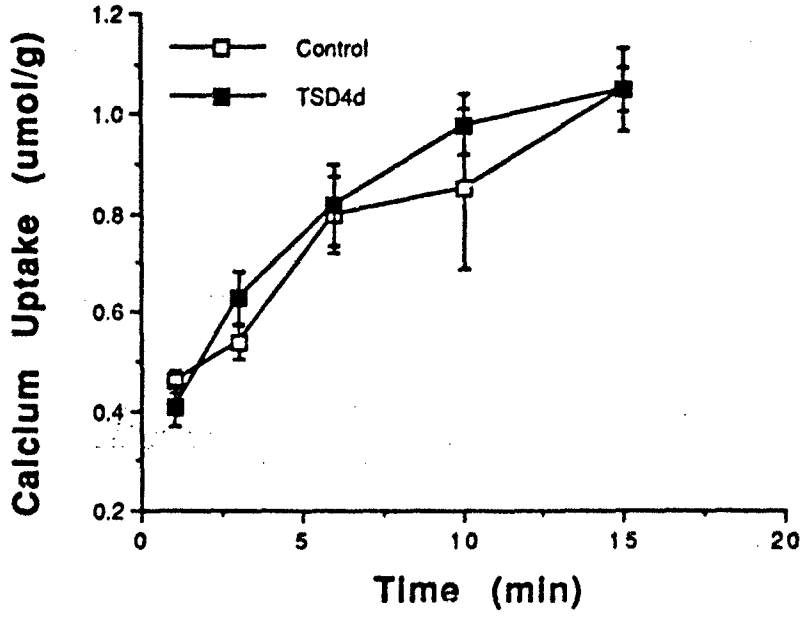

Fig. 66. The effects of ricin administration to rabbits on basal ^{45}Ca uptake in isolated aortas 4d after i.v. injection of a toxic sub-lethal dose of ricin. Each point is the mean ± S.E.M. of aorta pieces from 4 rabbits.

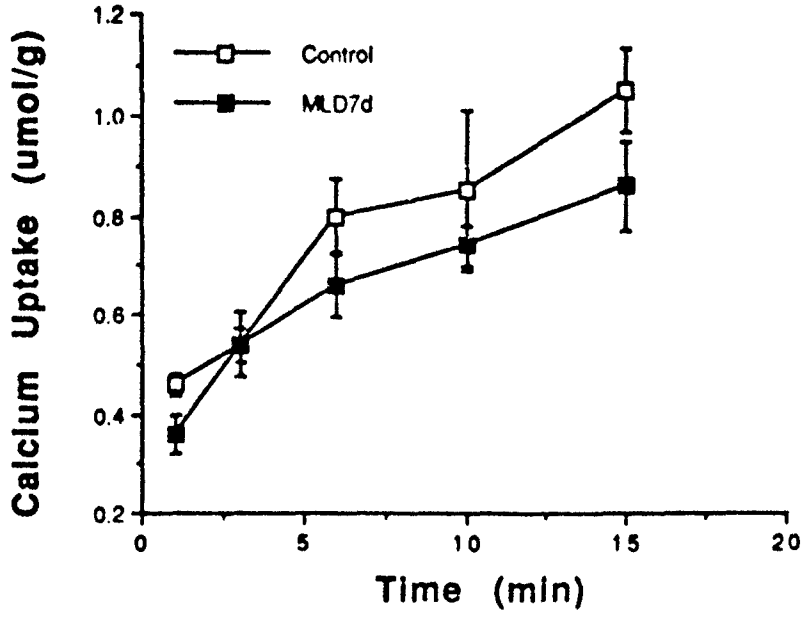

Fig. 67. The effects of ricin administration to rabbits on basal ^{45}Ca uptake in isolated aortas 7d after i.v. injection of a minimum lethal dose of ricin. Each point is the mean ± S.E.M. of aorta pieces from 4-6 rabbits.

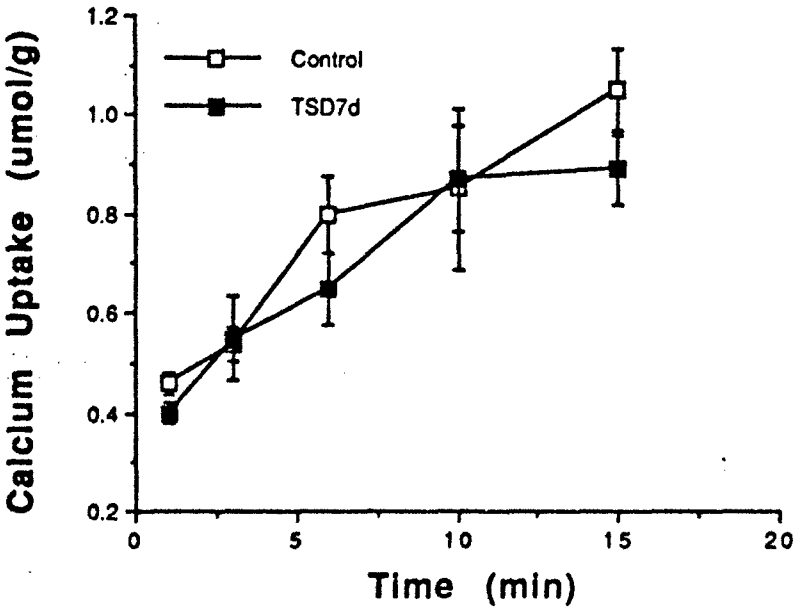

Fig. 68. The effects of ricin administration to rabbits on basal ^{45}Ca uptake in isolated aortas 7d after i.v. injection of a toxic sub-lethal dose of ricin. Each point is the mean ± S.E.M. of aorta pieces from 4-6 rabbits.

Table 22. The Effects of a Minimum Lethal Dose of Ricin Administration to Rabbits on KCl- or NE-Stimulated ^{45}Ca Uptake (μmol/g tissue) in Isolated Aortas.

^{45}Ca Uptake (μ mol/g tissue) Treated				
		Ricin Treated		
	Control	18h	4d	7d
80 mM KCl	3.31±0.07 (n=3)	1.24±0.04* (n=3)	3.17±0.81 (n=5)	1.93±0.46 (n=3)
100 μM NE	2.32±0.70 (n=4)	1.35±0.64 (n=3)	2.45±0.64 (n=5)	1.66±0.27 (n=5)

* Significantly different from control at $p < 0.05$.

Table 23. The Effects of a Toxic Sub-lethal Dose of Ricin Administration to Rabbits on KCl- or NE-Stimulated ^{45}Ca Uptake in Isolated Aortas.

^{45}Ca Uptake (μ mol/g tissue)				
		Ricin Treated		
	Control	18h	4d	7d
80 mM KCl	3.31±0.07 (n=3)	0.80±0.08* (n=4)	2.61±0.38 (n=4)	3.84±0.45 (n=4)
100 μM NE	2.32±0.70 (n=4)	1.05±0.11 (n=4)	1.77±0.38 (n=4)	2.37±0.43 (n=4)

* Significantly different from control at $p < 0.05$.

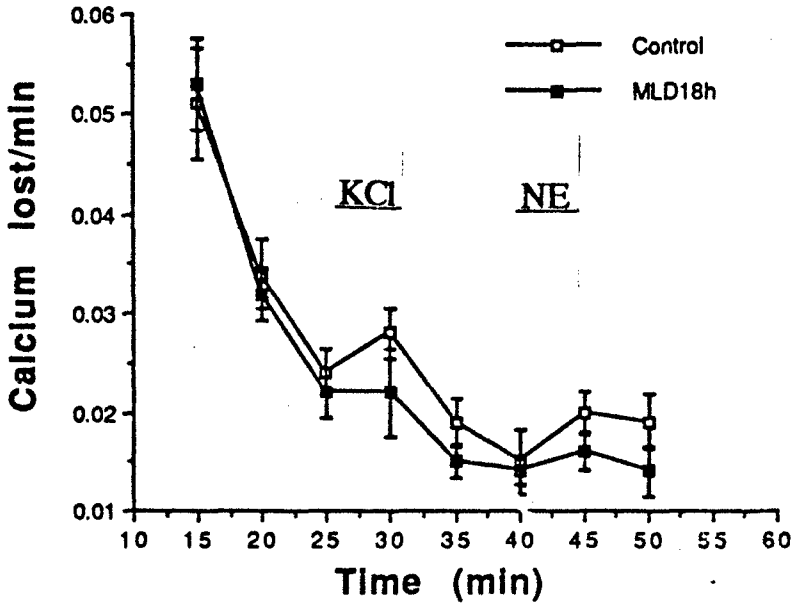

Fig. 69. The effects of ricin administration to rabbits on ^{45}Ca efflux in isolated aortas 18h after i.v. injection of a minimum lethal dose of ricin. 80 Mm KCl and 100 μM NE were in the contact solution for periods shown by the bars. Each point is the mean ± S.E.M. of 8-9 animals. The ^{45}Ca efflux rate is expressed as fraction of ^{45}Ca lost/min.

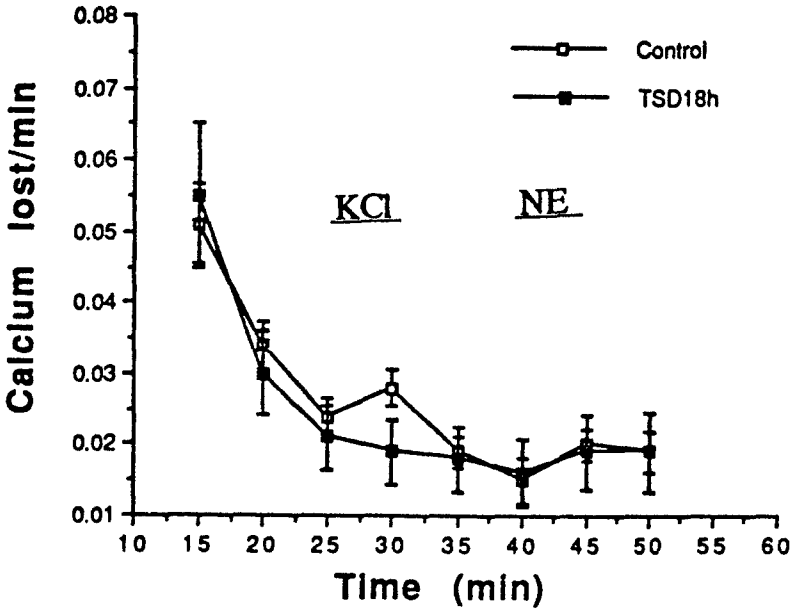

Fig. 70. The effects of ricin administration to rabbits on ^{45}Ca efflux in isolated aortas 18h after i.v. injection of a toxic sub-lethal dose of ricin. 80 Mm KCl and 100 μM NE were in the contact solution for periods shown by the bars. Each point is the mean ± S.E.M. of 5-9 animals. The ^{45}Ca efflux rate is expressed as fraction of ^{45}Ca lost/min.

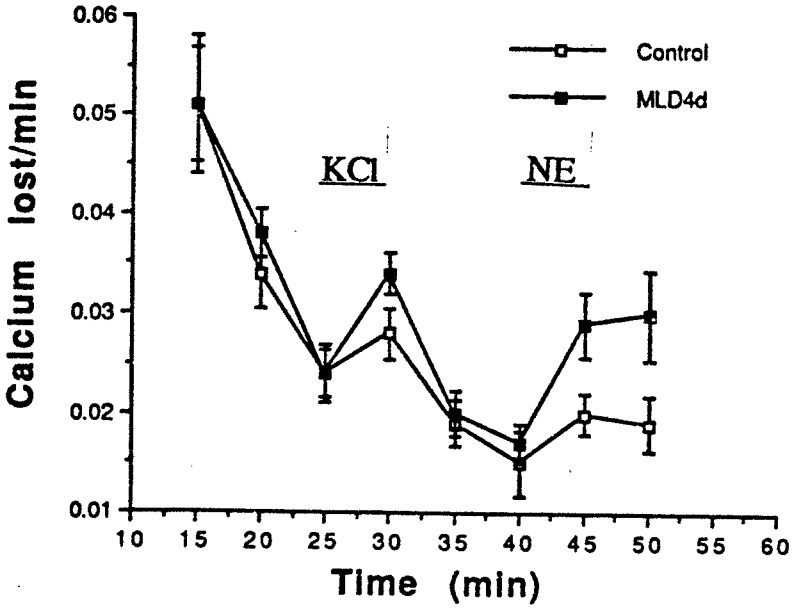

Fig. 71. The effects of ricin administration to rabbits on ^{45}Ca efflux in isolated aortas 4d after i.v. injection of a minimum lethal dose of ricin. 80 mM KCl and 100 μM NE were in the contact solution for periods shown by the bars. Each point is the mean ± S.E.M. of 6-9 animals. The ^{45}Ca efflux rate is expressed as fraction of ^{45}Ca lost/min.

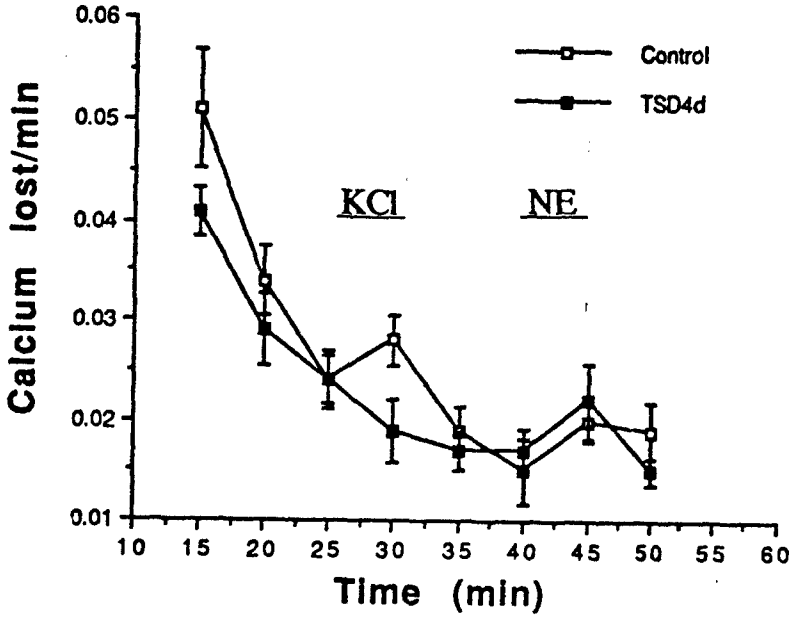

Fig. 72. The effects of ricin administration to rabbits on ^{45}Ca efflux in isolated aortas 4d after i.v. injection of a toxic sub-lethal dose of ricin. 80 mM KCl and 100 μM NE were in the contact solution for periods shown by the bars. Each point is the mean ± S.E.M. of 7-9 animals. The ^{45}Ca efflux rate is expressed as fraction of ^{45}Ca lost/min.

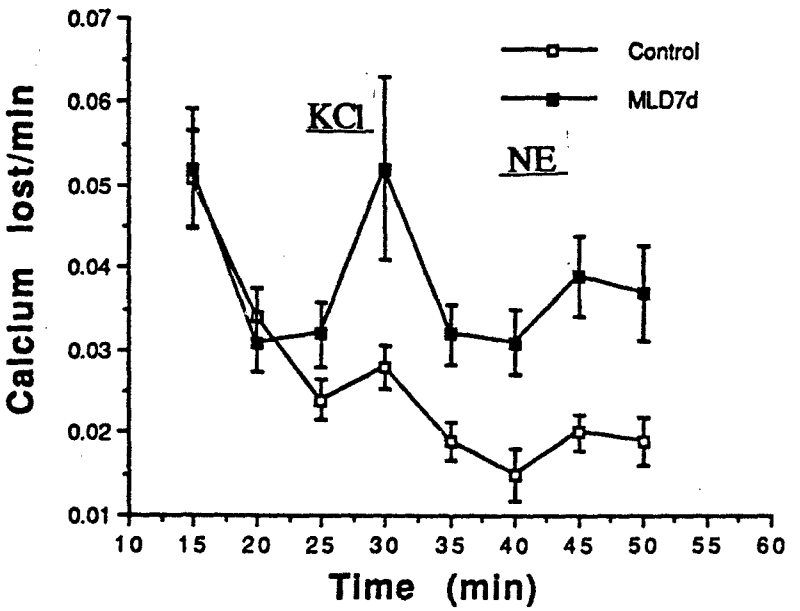

Fig. 73. The effects of ricin administration to rabbits on ^{45}Ca efflux in isolated aortas 7d after i.v. injection of a minimum lethal dose of ricin. 80 mM KCl and 100 μM NE were in the contact solution for periods shown by the bars. Each point is the mean ± S.E.M. of 6-9 animals. The ^{45}Ca efflux rate is expressed as fraction of ^{45}Ca lost/min.

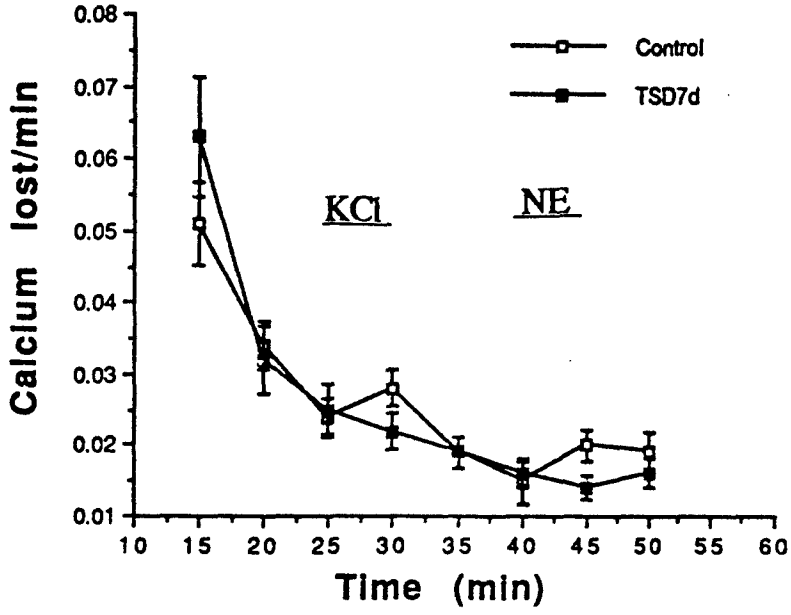

Fig. 74. The effects of ricin administration to rabbits on ^{45}Ca efflux in isolated aortas 7d after i.v. injection of a toxic sub-lethal dose of ricin. 80 mM KCl and 100 μM NE were in the contact solution for periods shown by the bars. Each point is the mean ± S.E.M. of 8-9 animals. The ^{45}Ca efflux rate is expressed as fraction of ^{45}Ca lost/min.

Table 24. The effects of the minimum lethal dose or toxic sub-lethal dose of ricin on 80 mM KCl-stimulated ^{45}Ca efflux (expressed as fraction of ^{45}Ca lost/min) from isolated rabbit aortas.

RICIN DOSE	TIME PERIOD FROM RICIN ADMINISTRATION TO OBTAINING TISSUES			
		18h	4d	7d
	Mean±S.E.M. (n)	Mean±S.E.M. (n)	Mean±S.E.M. (n)	Mean±S.E.M. (n)
Control	0.028±0.005* (9)			
Toxic Sub-lethal Dose		0.0191±0.005* (5)	0.019±0.003*+ (7)	0.022±0.003* (8)
Minimum Lethal Dose		0.022±0.005* (8)	0.034±0.002 (6)	0.052±0.011 (6)

* Significantly different from MLD 7d at $p < 0.05$.
\+ Significantly different from MLD 4d at $p < 0.05$.

Table 25. The effects of the minimum lethal dose or toxic sub-lethal dose of ricin on 100 μM NE-stimulated ^{45}Ca efflux (expressed as fraction of ^{45}Ca lost/min) from isolated rabbit aortas.

RICIN DOSE	TIME PERIOD FROM RICIN ADMINISTRATION TO OBTAINING TISSUES			
		18h	4d	7d
	Mean±S.E.M. (n)	Mean±S.E.M. (n)	Mean±S.E.M. (n)	Mean±S.E.M. (n)
Control	0.020±0.002* (9)			
Toxic Sub-lethal Dose		0.019±0.005* (6)	0.022±0.004* (6)	0.014±0.002* (8)
Minimum Lethal Dose		0.016±0.002* (8)	0.029±0.003 (5)	0.039±0.005 (6)

* Significantly different from MLD 4d and 7d at $p < 0.05$.

IV. DISCUSSION AND CONCLUSIONS

Both the minimal lethal dose and the toxic sublethal dose lowered both systolic and diastolic pressures, but only the minimum lethal dose did so significantly. There was no consistent effect of either dose on blood pressure during the first 12 hours. Only after about 22-28 hours post-ricin injection with the minimum lethal dose were the depressor effects on blood pressure marked. In man, ricin also decreases blood pressure (Balint, 1974).

It seems likely that the effects of ricin on heart rate were at least primarily reflex adjustments due to baroreceptor reflexes. Evidence for this is the reciprocal relationship between the heart rate and blood pressure. In the first few hours and in the last approximately 24 hours of the 12-48 hour post-ricin observation period, blood pressure was reduced and heart rate was elevated. In man, ricin also increases heart rate (Balint, 1974).

The ECG was not altered during the observation period.

Abnormal laboratory values correlated well with histological findings. Both serum CPK and LDH were elevated. This could occur from damage to the heart muscle, which was observed histologically. The elevated SGPT observed is indicative of liver damage, which was also observed histologically. As elevated LDH is observed in both myocardial and hepatic damage, the increased concentrations of this enzyme may have resulted from damage to either or both of these organs.

Serum cholesterol concentrations were markedly increased following ricin administration. There are many possible causes of ricin-induced abrupt rises in circulating cholesterol concentrations. These include, among others: increased cholesterol synthesis, decreased conversion of cholesterol to bile acids, and damage to cell membranes comprising blood vessels (i.e., endothelial and smooth muscle cells) or other cells, releasing cholesterol from the membranes. Cholesterol is an important lipid component of cell membranes. When the lipid content of human erythrocyte membranes is reduced in vitro, the uptake of calcium through voltage dependent calcium channels is reduced (Locher *et al.*, 1984). It is interesting to observe that if ricin damages cell membranes in vascular smooth muscle with loss of some cholesterol from the membranes, it could cause the reduction through voltage dependent calcium uptake calcium channels which we observed following ricin administration.

Serum calcium concentrations were lower in rabbits given ricin. The reason

for this reduction is not known. Because calcium concentrations are controlled within a narrow range normally, and this is important for proper function, this may be an important toxic effect.

It seems that rabbits that die early (approximately 22 hours after the i.v. injection of ricin) have marked pulmonary damage. Asthmatic symptoms were observed, which would be caused by the pulmonary damage that we observed histologically (Balint, 1974). Those that die later, i.e. about 36 or 48 hours after ricin administration, have much more heart and liver damage as well as damage to other organs. The pathological changes in the liver, necrosis of the centrilobular and paracentral zones with sparing of the periportal zone, is typical of the damage observed in congestive heart failure or other conditions where there is inadequate perfusion of the liver rather than a direct toxic effect of ricin. The blood flow studies indicate no decrease in blood flow to the liver, but these studies were only done up to 18 hours post injection and damage to the liver did not occur in rabbits which died at 24 hours, only in those that died at 36 or 48 hours post injection. The severe hemorrhage observed in microscopic examination of the myocardium may indicate a decrease in function of the heart muscle. This would be consistent with the hypothesis that the damage observed in the liver is secondary to inadequate perfusion rather than a direct toxic effect of ricin. In man, post-mortem degenerative changes were also noted in the liver and heart. Also degenerative changes in lymphoid tissues and stomach were found both in our studies, and in humans following ricin poisoning (Balint, 1974).

One area in which our microscopic examination of the tissue failed to identify tissue damage and yet flow was markedly altered was the brain. The lower ricin dose increased blood flow by 147% at 12 hours, but by 18 hours blood flow was down to only 4% above control. With the minimum lethal dose, blood flow was markedly decreased at both time periods, by 35% at 12 hours and by 50% at 18 hours.

Thus, ricin increases total coronary output by approximately 30%, but alterations of blood flow to individual tissues vary markedly. In some tissues, the lower ricin dose increases blood flow while the minimum lethal dose decreases blood flow, at least at 18 hours.

In the studies of ricin's effects on blood flow, cardiac output was increased at both 12 and 18 hours after ricin injection. Because blood pressure was not altered during this period, vasodilation was also occurring.

The increased cardiac output (about 30% with each of the time points) was not evenly distributed among all of the tissues. Among the tissues in which blood flow was increased the most was the heart, with coronary perfusion increased by 127 and 150% at 12 and 18 hours after a toxic sub-lethal dose, respectively, and by 180 and 205% at 12 and 18 hours, respectively, after a minimum lethal dose. This increased blood flow would result in increased ricin delivery to the myocardium. The heart was one of the areas where histological evidence of tissue damage was identified.

Another area of tissue damage was the lungs. In the lungs, a toxic sub-lethal dose increased blood flow by 13 and 181% at 12 and 18 hours, respectively, while the minimum lethal dose increased blood flow by only 3% at 12 hours, and decreased it by 52% at 18 hours. Perhaps at this higher ricin dose, tissue damage may have already been occurring which caused the reduction in blood flow.

In our studies comparing the contractions of central ear arteries to NE in ricin treated and control rabbits, we found a decrease in sensitivity to NE with no significant change in maximal contraction. NE stimulates phospholipase C in smooth muscle cells, and in doing so, releases arachidonic acid as well as diacylglycerol and inositol 1,4,5-triphosphates (IP_3) (Martin and Wysolmerski, 1987). Ricin increases production of arachidonic acid metabolites in macrophages (Naseem and Pace, 1991). Prostacyclin, a relaxant prostaglandin, is the major metabolic product of arachidonic acid formed by cyclooxygenase in smooth muscle cells (Shepherd & Kutusic, 1991). Arachidonic acid released following exposure to NE may then be metabolized to relaxant prostaglandins, such as prostacyclin, to a greater extent following ricin administration. Therefore, the increased relaxant prostaglandins formed may be functioning as physiological antagonists to NE-induced contractions, resulting in a decreased sensitivity of the tissue to NE.

Our studies show an increase in relaxation to methacholine in NE contracted aortas from ricin treated rabbits. Methacholine elicits a relaxation by acting on muscarinic receptors to release endothelial-derived relaxing factor(s) (Furchgott and Zawadski, 1980a; Furchgott, 1981). The increase in response to methacholine following ricin administration could be a result of increased release of EDRF. Histamine and bradykinin cause a rapid increase in intracellular calcium and inositol phosphate concentrations in endothelial cells in cell culture (Rotrosen and Gallin, 1986; Derian and Moskowitz, 1986; Lambert *et al.*, 1986; Colden-Stanfield *et al.*, 1987). The release of EDRF is dependent upon the influx of calcium (Singer and Peach, 1982) and is stimulated by A23187, a calcium ionophore. Nitric oxide, which has been proven to be EDRF in many tissues, is formed by the action of NO

synthase. This is a monooxygenase which is activated by increased intracellular concentrations of calcium (Ignarro, 1990). Therefore, the increased intracellular calcium in endothelial cells produced by histamine and bradykinin could result in an increased production and release of endothelial-derived relaxing factor(s). Ricin administration produces many allergic and hypersensitivity type reactions that are indicative of histamine release (Balint, 1974). The release of histamine by ricin causing an influx of calcium into the endothelial cells and resulting in a release of endothelial-derived relaxing factor(s) is consistent with labilizing of the endothelium by ricin.

The increased relaxation observed in our studies was temporary since the relaxations were returning toward control at 4 and 7 days. Since histamine release likely decreases after initial tissue damage by ricin, a decreased enhancement of methacholine-induced relaxations at four and seven days after ricin administration would be expected. Thus our results are consistent with the hypothesis that the release of histamine by ricin caused an increased intracellular calcium concentration in endothelial cells leading to the increased release of EDRF.

Nassem and Pace (1991) found that macrophages incubated with ricin, release increased arachadonic acid metabolites compared to control. Because endothelium-derived relaxants may in some cases be prostaglandins (Furchgott and Zawadzki, 1980a; Furchgott, 1983; Singer and Peach, 1983; DeMey *et al.*, 1983; Singer *et al.*, 1984; Pinto *et al.*, 1986), it is possible that increased synthesis of one or more relaxant prostaglandins may be involved in the effects of ricin administration on the enhanced relaxations to methacholine.

Another mechanism by which aorta rings from rabbits given ricin may have enhanced endothelium-dependent relaxations could be by increasing concentrations of the NO precursor arginine within the endothelial cells. Because ricin decreases protein synthesis (Olsnes *et al.*, 1974; Endo *et al.*, 1987), there may be more free amino acids inside endothelial cells. If arginine concentrations are increased within the endothelial cells this could result in an increased NO formation, and enhance endothelium dependent relaxations, as we observed in our studies with the aorta.

In the rabbit aorta, ATP exerts most of its relaxant effects through an endothelium dependent mechanism (Furchgott and Zawadski, 1980b; Furchgott, 1981). There is a component of the relaxation to ATP that is endothelium independent, mediated by the metabolites AMP and adenosine at purinergic receptors. In some studies we used the aorta to investigate relaxations. We found decreased relaxations to ATP in the aorta. This may not be inconsistent with

enhanced endothelium dependent effects, as the depression of the effect of ATP in the aorta may be more due to alteration of the purinergic receptor or coupling mechanisms. Total relaxations to the concentration of ATP we used were small, however, and this may not have much significance in toxic effects of ricin in the rabbit.

Relaxations to papaverine in the NE contracted aorta were not altered by ricin administration. This indicates that the ability of the artery to relax to a non-specific agonist is not impaired by ricin administration.

Norepinephrine efflux to transmural stimulation was increased following ricin administration in some cases. NE uptake was not altered by ricin administration. Therefore, an increased efflux is indicative of an increased NE release from sympathetic nerves. NE content in aorta was higher in five of six ricin treated groups compared to control, although only one was significantly higher. An increased NE content could partially explain the increased NE release following ricin administration. An increased NE release could also result from compensatory changes due to a decreased sensitivity of the post-synaptic receptor to NE or alterations of excitation-contraction coupling mechanisms.

Tyramine releases NE from sympathetic nerves in the central ear artery. Contractions to tyramine were not altered by ricin administration despite the fact that sensitivity of the ear artery to NE had decreased. An increased release of NE by tyramine (which would tend to cause an increased contraction) opposed by a decreased sensitivity of the ear artery to NE (which would tend to decrease contractions) could result in an unchanged tyramine induced contraction. This seems to indicate that ricin administration not only increases NE release following transmural stimulation, but also following tyramine administration.

Since MAO and COMT activities were not significantly altered following ricin treatment, changes in neurotransmitter metabolism are not involved in the toxicity of ricin.

Norepinephrine-stimulated calcium uptake was reduced (but not significantly) at 18 hours following both ricin doses, and was returning toward control thereafter. Although these results indicate that depression of the function of receptor-operated calcium channels was not significant, this depression would be consistent with the results of our contraction experiments in the central ear artery. The increased EC_{50} (decreased sensitivity) might be a result of a decreased influx of calcium following exposure to NE.

Potassium stimulated calcium influx was significantly depressed by both ricin doses at 18 hours. This indicates that the opening of voltage-dependent calcium channels is depressed by ricin treatment. This depression was reversible as indicated by the increased calcium influx at the next two time periods. The results may partially explain the decreased sensitivity of central ear arteries from ricin treated rabbits to NE-induced contractions as NE also opens voltage-dependent Ca^{2+} channels in vascular smooth muscle (van Breemen and Saida, 1989). The results may also partially explain the increased cardiac output and blood flow to most tissues in rabbits receiving ricin. Since voltage-dependent Ca^{2+} channels are numerous in most resistance arterioles (van Breemen and Saida, 1989), a depression of calcium influx would lead to relaxation of the vessels increasing blood flow to the tissues.

The MLD of ricin increases NE-stimulated calcium efflux from the rabbit aorta. This indicates that IP_3-induced calcium release from intracellular stores is enhanced by administration of the MLD of ricin. The MLD of ricin also increases potassium-stimulated calcium efflux, indicating that calcium-induced calcium release is also enhanced by administration of an MLD of ricin. The effects on calcium efflux are slowly developing, indicating that protein synthesis inhibition may be involved in the efflux alteration.

In summary ricin administration to rabbits causes pathological changes in the heart, liver, lungs and other tissues. It increases enzymes indicative of heart and liver damage and alters certain other laboratory values. It decreases blood pressure after a lag period of 20-24 hours, and increases blood flow to most tissues at 18 or 24 hours post injection.

Underlying mechanisms for these changes in blood pressure and blood flow include a decreased sensitivity to alpha-adrenergic receptor stimulation in blood vessels, enhanced endothelium dependent arterial relaxations, and decreased calcium influx through voltage-dependent calcium channels.

Our suggestions for future areas of research into ricin's toxic effects include in depth studies on the heart and coronary arteries, studies of toxic effects on the lungs and lung preparations, and studies of ricin's effects on the vascular endothelium to include studies in cell culture.

V. ACKNOWLEDGMENTS

The authors wish to thank:

- Donald Parker, Ph.D., University of Oklahoma, Department of Biostatistics and Epidemiology, for his help with statistical analyses.
- Lianmin Ma, M.D., cardiologist, for evaluating the ECG tracings.
- Garo P. Basmadjian, Ph.D., and Stanley L. Mills, Ph.D., for assistance with radioactivity determinations in the studies with radiolabeled microspheres.
- Stanley D. Kosanke, DVM, Ph.D., Department of Pathology, College of Medicine, University of Oklahoma, for preparation and evaluation of tissue slides.
- Leslie Patatanian, Steve Boatright, Jodie Cardenas, and Chanda Desbien for technical assistance and help with the statistical analyses.
- Paula A. Meder for typing this document.

VI. REFERENCES

1. Araki, T. and Funatsu, G. Revised amino acid sequence of the B-chain of ricin D due to loss of tryptophan in the cyanogen bromide cleavage. FEBS Letters 191:121-124, 1985.

2. Araki, T. and Funatsu, G. The complete amino acid sequence of the B-chain of ricin E isolated from small-grain castor bean seeds. Ricin E is a gene recombination product of ricin D and Ricinus communis agglutinin. Biochim. Biophys. Acta 911:191-200, 1987.

3. Araki, T., Yoshioka, Y. and Funatsu, G. The complete amino acid sequence of the B-chain of the Ricinus communis agglutinin isolated from large-grain castor bean seeds. Biochim. Biophys. Acta 872:277-285, 1986.

4. Axelrod, J. Catechol-*O*-methyltransferase from rat liver, in Methods in Enzymology (S.P. Colowick and N.O. Kaplan, eds.) Vol. 5, pp. 748-749, Academic Press, New York, 1962.

5. Baenziger, J.U. and Fiete, D. Structural determinants of Ricinus Communis agglutinin and toxin specificity of oligosaccharides. J. Biol. Chem. 254:9795-9799, 1979.

6. Balint, G.A. Ricin: The toxic protein of castor oil seeds. Toxicol. 2:77-102, 1974.

7. Bavari, S., Walters, D.M. and Creasia, D.A. Ricin and ricin B chain stimulate the release of tumor necrosis factor-alpha from alveolar macrophages. The Toxicologist 12:Abstract 1126, 1992.

8. Blakey, D. and Thorpe, P. Treatment of malignant disease and rheumatoid arthritis using ricin A-chain immunotoxins. Scand. J. Rheumatology Supp. 76:279-287, 1988.

9. Blakey, D., Watson, G., Knowles, P. and Thorpe, P. Effect of chemical deglycosylation of ricin A-chain on the in vivo fate and cytotoxic activity of an immunotoxin composed of ricin A-chain and anti-Thy 1.1 antibody. Cancer Res. 47:947-952, 1987.

10. Bourrie, B., Casellas, P., Blythman, H. and Jansen, F. Study of the plasma clearance of antibody-ricin A-chain immunotoxins. Evidence for specific recognition sites of the A-chain that mediate rapid clearance of the immunotoxin. Eur. J. Biochem. 155:1-10, 1986.

11. Byers, V., Henslee, P., Kernan, N., Blazar, B., Gingrich, R., Phillips, G., LeMaistre, C., Gilliland, G., Antin, J., Martin, P., Tutscha, P., Trown, P., Ackerman, S., O'Reilly, R. and Scannon, P. Use of an anti-pan T-lymphocyte ricin A chain immunotoxin in steroid-resistant acute graft-versus-host disease. Blood 75:1426-1432, 1990.

12. Cawley, D.B. and Houston, L.L. Effect of sulfhydryl reagents and protease inhibitors on sodium dodecyl sulfate-heat induced dissociation of Ricinus Communis agglutinin. Biochim. Biophys. Acta 581:51-62, 1979.

13. Clarke, E.G.C. The detection of ricin. J. Pharm. Pharmacol. 5:458-459, 1953.

14. Cmiech, H.A., Morley, M. and Gee, D.J. Immunocytochemical detection of ricin. I. Preliminary immunofluorescence studies. Histochem. J. 17:859-866, 1985.

15. Colden-Stanfield, M., Schilling, W.P., Ritchie, A.K., Eskin, S.G., Navarro, L.T. and Kunze, D.L. Bradykinin-induced increases in cytosolic calcium and ionic currents in cultured bovine aortic endothelial cells. Circ. Res. 61:632-640, 1987.

16. Colombatti, M., Colombatti, A., Blythman, H.E. and Bron, C. Thy 1.2^{+} leukemia cells eradicated from in vitro leukemia-bone marrow cell mixtures by antibody-toxin conjugates. JNCI 72:1095-1099, 1984.

17. Creasia, D.A., Bavari, S., Bostian, K.A. and Walters, D.M. Broncho-alveolar lavage fluid enzymes after exposure of mice to ricin and ricin B chain aerosols. The Toxicologist 12:Abstract 1125, 1992.

18. Crompton, R. and Gall, D. Georgi Markov - Death in a pellet. Medico-Legal J. 48:51-62, 1980.

19. De Mey, J.G. and Vanhoutte, P.M. Anoxia and endothelium-dependent reactivity of the canine femoral artery. J. Physiol. 335:65-74, 1983.

20. Derenzini, M., Benetti, E., Marinozzi, V. and Stirpe, F. Toxic effects of ricin: Studies on the pathogenesis of liver lesions. Virchows Arch. B 20:15-28, 1987.

21. Derian, C.K. and Moskowitz, M.A. Polyphosphoinositide hydrolysis in endothelial cells and carotid artery segments. J. Biol. Chem. 261:3831-3837, 1986.

22. Dixon, W.J. The Up-and-down method for small samples. Am. Stat. Assoc. J. 60:967-984, 1965.

23. Ehrlich, P. The collected papers of Paul Ehrlich, vol. 2 pp.21-44. 1957. New York:Pergamon.

24. Eiklid, K., Olsnes, S. and Pihl, A. Entry of lethal doses of abrin, ricin and modeccin into the cytosol of HeLa cells. Exp. Cell Research 126:321-326, 1980.

25. Endo, Y., Mitsui, K., Motizuki M. and Tsurugi, K. The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes. J. Biol. Chem. 262:5908-5912, 1987.

26. Endo, Y. and Tsurugi, K. RNA N-glycosidase activity of ricin A-chain. J. Biol. Chem. 262:8128-8130, 1987.

27. Engert, A., Burrows, F., Jung, W., Tazzari, P., Stein, H., Pfreundschuh, M., Diehl, V. and Thorpe, P. Evaluation of ricin A chain-containing immunotoxins directed against the CD30 antigen as potential reagents for the treatment of Hodgkin's disese. Cancer Res. 50:84-88, 1990a.

28. Engert, A., Martin, G., Pfreundschuh, M., Amlot, P., Hsu, S.-M., Diehl, V. and Thorpe, P. Antitumor effects of ricin A chain immunotoxins prepared from intact antibodies and fab' fragments on solid human Hodgkin's disease tumors in mice. Cancer Res. 50:2929-2935, 1990b.

29. Ettenson, D., Sheldon, K., Marks, A., Houston, L. and Baumal, R. Comparison of growth inhibition of a human ovarian adenocarcinoma cell line by free monoclonal antibodies and their corresponding antibody-recombinant ricin A chain immunotoxins. Anticancer Res. 8:833-838, 1988.

30. FitzGerald, D., Bjorn, M., Ferris, R., Winkelhake, J., Frankel, A., Hamilton, T., Ozols, R., Willingham, M. and Pastan, I. Antitumor activity of an immunotoxin in a nude mouse model of human ovarian cancer. Cancer Res. 47:1407-1410, 1987.

31. Fodstad, O., Johannessen, J.V., Schjerven, L. and Pihl, A. Toxicity of abrin and ricin in mice and dogs. J. Toxicol. Environ. Health. 5:1073-1084, 1979.

32. Fodstad, O., Kvalheim, G., Godal, A., Lotsberg, J., Aamdal, S., Host, H., and Pihl, A. Phase I study of the plant protein ricin. Cancer Res. 44:862-865, 1984.

33. Fodstad, O., Olsnes, S. and Pihl, A. Toxicity, distribution and elimination of the cancerostatic lectins abrin and ricin after parenteral injection into mice. Br. J. Cancer. 34:418-425, 1976.

34. Fodstad, O. and Pihl, A. Effect of ricin and abrin on survival of L1210 leukemic mice and on leukemic and normal bone marrow cells. Int. J. Cancer 22:558-563, 1978.

35. Fodstad, O. and Pihl, A. Synergistic effect of Adriamycin and ricin on L1210 leukemic cells in mice. Cancer Res. 40:3735-3739, 1980.

36. Foxwell, B.M.J., Detre, S.I., Donovan, T.A. and Thorpe, P.E. The use of anti-ricin antibodies to protect mice intoxicated with ricin. Toxicology 34:79-88, 1985.

37. Fulton, R.J., Uhr, J.W. and Vitetta, E.S. In vivo therapy of the BCL_1 tumor: effect of immunotoxin valency and deglycosylation of the ricin A chain. Cancer Res. 48:2626-2631, 1988.

38. Funatsu, M., Funatsu, G., Ishiguro, M. and Hara, K. Properties of subunits of ricin D. Jap. J. Med. Sci. Biol. 26:30-32, 1973.

39. Funatsu, M., Funatsu, G., Ishiguro, S., Nanno, S. and Hara, K. Structure and toxic function of ricin. I. Purification procedures of ricin D. II. Subunit structure of ricin D. Proc. Jap. Acad. 47:713-723, 1971.

40. Funatsu, G., Kimura, M. and Funatsu, M. Primary structure of Ala chain of ricin D. Agric. Biol. Chem. 43:2221-2224, 1979.

41. Funatsu, G., Yoshitake, S. and Funatsu, M. Primary structure of Ile chain of ricin D. Agric. Biol. Chem. 42:501-503, 1978.

42. Furchgott, R.F. The requirement for endothelial cells in the relaxation of arteries by acetylcholine and some other vasodilators. Trends Pharmacol. Sci. 2:173-176, 1981.

43. Furchgott, R.F. Role of endothelium in responses of vascular smooth muscle. Circ. Res. 53:557-573, 1983.

44. Furchgott, R.F. and Zawadzki, J.V. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373-376, 1980a.

45. Furchgott, R.F. and Zawadzki, J.V. ATP relaxes rabbit aortic smooth muscle by both an indirect action via endothelial cells and a direct action. Pharmacologist 22:271, 1980b.

46. Furutani, M., Kashiwagi, K., Ito, K., Endo, Y. and Igarashi, K. Comparison of the modes of action of a vero toxin (a shiga-like toxin) from *Escherichia coli*, of ricin, and of α-sarcin. Archives of Biochem. & Biophysics 293:140-146, 1992.

47. Godal, A., Fodstad, O., Ingebrigtsen, K. and Pihl, A. Pharmacological studies of ricin in mice and humans. Cancer Chem. Pharmacol. 13:157-163, 1984.

48. Godal, A., Fodstad, O. and Pihl, A. Antibody formation against the cytotoxic proteins abrin and ricin in humans and mice. Int. J. Cancer 32:515-521, 1983.

49. Godfraind, T., Miller, R.C. and Socrates Lima, J. Selective α_1- and α_2-adrenoceptor agonist-induced contraction and ^{45}Ca fluxes in the rat isolated aorta. Br. J. Pharmacol. 77:597-604, 1982.

50. Goldmacher, V.S., Lambert, J.M. and Blättler, W.A. The specific cytotoxicity of immunoconjugates containing blocked ricin is dependent on the residual binding capacity of blocked ricin: evidence that the membrane binding and A-chain translocation activities of ricin cannot be separated. Biochem. Biophys. Res. Comm. 183:758-766, 1992.

51. Gregg, E., Bridges, S., Youle, R., Longo, D., Houston, L., Glennie, M., Stevenson, F. and Green, I. Whole ricin and recombinant ricin A chain idiotype-specific immunotoxins for therapy of the guinea pig L_2C B cell leukemia. J. Immunology 138:4502-4508, 1987.

52. Griffin, T., Richardson, C., Houston, L., LePage, D., Bodgen, A. and Raso, V. Antitumor activity of intraperitoneal immunotoxins in a nude mouse model of human malignant mesothelioma. Cancer Res. 47:4266-4270, 1987.

53. Griffin, T., Pganini, P., McGrath, J., McCann, J. and Houston, L. In vitro cytotoxicity of recombinant ricin A chain-antitransferrin receptor immunotoxin against human adenocarcinomas of the colon and pancreas. J. Bioi. Resp. Mod. 7:559-567, 1988.

54. Griffith, S.G., Crowe, R., Lincoln, J., Haven, A.J. and Burnstock, G. Regional differences in the density of perivascular nerves and varicosities, norepinephrine content and responses to nerve stimulation in the rabbit ear artery. Blood Vessels 19:41-52, 1982.

55. Griffiths, G., Leek, M. and Gee, D. The toxic plant proteins ricin and abrin induce apoptotic changes in mammalian lymphoid tissues and intestine. J. Pathol. 151:221-229, 1987.

56. Griffiths, G.D., Leith, A.G., Leek, M.D. and Green, M.A. Demonstration of ricin within the mammalian para-aortic lymph node: I. comparison of the localization, after intramuscular injection, with three immunocytochemical methods. Histochem. J. 21:380-386, 1989a.

57. Griffiths, G.D., Leith, A.G., Leek, M.D. and Green, M.A. Distribution of ricin within the mammalian para-aortic lymph node: II. comparison of the localization, after intramuscular dosage of colloidal gold-labelled ricin in vivo, with in vitro binding characteristics of the native toxin. Histochem. J. 21:387-392, 1989b.

58. Griffiths, G.D., Newman, H.V. and Gee, D.J. Identification and quantification of ricin toxin in animal tissues using ELISA. J. Forensic. Sci. Soc. 26:349-358, 1986a.

59. Griffiths, G.D., Newman, H.V. and Gee, D.J. Immunocytochemical detection of ricin. II. Further studies using the immunoperoxidase method. Histochemical J. 18:189-195, 1986b.

60. Hales, J.R.S. Radioactive microsphere techniques for studies of the circulation. Clin. Exp. Pharmacol. Physiol. Suppl, 31-46, 1974.

61. Hansen, S., Petersen, O., Sandvig, K., Olsnes, S. and van Deurs, B. Internalized ricin and the plasma memebrane glycoprotein MAM-6 colocalize in the trans-golgi network of T47D human breast carcinoma cells. Exp. Cell Res. 185:373-386, 1989.

62. Harper, C.G., Gonatas, J.O., Mizutani, T. and Gonatas, N.K. Retrograde transport and effects of toxic Ricin in the autonomic nervous system. Lab. Invest. 42: 396-404, 1980.

63. Hatakeyama, T., Ohba, H., Yamasaki, N. and Funatsu, G. Binding of saccharides to ricin E isolated from small castor beans. J. Biochem. 105:444-448, 1989.

64. Hatakeyama, T., Yamzasaki, N. and Funatsu, G. Further studies on the histidine residue located at the high affinity saccharide-binding site of ricin E. Agric. Biol. Chem. 54:1085-1086, 1990.

65. Helke, C., Charlton, C. and Wiley, R. Suicide transport of ricin demonstrates the presence of substance P receptors on medullary somatic and autonomic motor neurons. Brain Res. 328:190-195, 1985.

66. Hertler, A., Schlossman, D., Borowitz, M., Laurent, G., Jansen, F., Schmidt, C. and Frankel, A. A Phase I study of T101-ricin A chain immunotoxin in refractory chronic lymphocytic leukemia. J. Biol. Resp. Mod. 7:97-113, 1988.

67. Hewetson, J.F., Rivera, V.R., Creasia, D.A., Lemley, P.V., Rippy, M.K. and Poli, M.A. Protection of mice from inhaled ricin exposure by vaccination with ricin or by passive treatment with heterologous antibody. FASEB J. 5:Abstract 795, 1991.

68. Hirio, M., Sekizawa, T. and Openshaw, H. Ricin injection eliminates latent herpes simplex virus in the mouse. J. Infect. Dis. 157:1270-1271, 1988.

69. Ignarro, L.J. Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu. Rev. Pharmacol. Toxicol. 30:535-560, 1990.

70. Ishiguro, M., Mitarai, M., Harada, H., Sekine, I., Nishimori, I. and Kikutani, M. Biochemical studies on oral toxicity of ricin. I. ricin administered orally can impair sugar absorption by rat small intestine. Chem. Pharm. Bull. 31:3222-3227, 1983.

71. Ishiguro, M., Nakashima, H., Tanabe, S. and Sakakibara, R. Interaction of toxic lectin ricin with epithelial cells of rat small intestine *in vitro*. Chem. Pharm. Bull. 40:441-445, 1992.

72. Janis, R.A., Silver, P.J. and Triggle, D.J. Drug action and cellular calcium regulation. Advances in Drug Res., 16:309-591, 1987.

73. Janossy, G., Panayi, G., Duke, O., Bofill, M., Poulter, L. and Goldstein, G. Rheumatoid arthritis, a disease of LT-lymphocyte/macrophage immunoregulation. Lancet ii:839-842, 1981.

74. Kanellos, J., MacKenzie, I. and Pietersz, G. In vivo studies of whole ricin monoclonal antibody immunoconjugates for the treatment of murine tumours. Immunol. Cell Biol. 66:403-415, 1988.

75. Kanellos, J., MacKenzie, I. and Pietersz, G. Intratumour therapy of solid tumours with ricin-antibody conjugates. Immunol. Cell Biol. 67:89-99, 1989.

76. Katzin, B.J., Collins, E.J. and Robertus, J.D. Structure of ricin A-chain at 2.5 Å. Proteins: Structure, Function, and Genetics 10:251-259, 1991.

77. Keller, R., Oke, A., Mefford, I. and Adams, R.N. Liquid chromatographic analysis of catecholamine (routine assay for regional brain mapping). Life Sci. 19:995-1004, 1976.

78. Ko, T. and Kaji, A. Effect of ricin on biosynthesis of myeloma protein (IgA) and general cellular proteins in MOPC-315 cells. Biochim. Biophys. Acta 414:155-160, 1975.

79. Koga, M., Ohtsu, M. and Funatsu, G. Cytotoxic, cell agglutinating, and syncytium forming effect of purified lectins from Ricinus Commucin on cultured cells. Gann 70:585-591, 1979.

80. Koja, N., Shibata, T. and Mochida, K. Enzyme-linked immunoassay of ricin. Toxicon 18:611-618, 1980.

81. Kopferschmitt, J., Flesch, F., Lugnier, A., Sauder, P.H., Jaeger, A. and Mantz, J.M. Acute voluntary intoxication by ricin. Human Toxicol. 2:239-242, 1983.

82. Krolick, K.A., Uhr, J.W. and Vitetta, E.S. Selective killing of leukaemia cells by antibody-toxin conjugates: implications for autologous bone marrow transplantation. Nature 295:604-605, 1982.

83. Krönke, M., Depper, J.M., Leonard, W.J., Vitetta, E.S., Waldmann, T.A. and Greene, W.C. Adult T cell leukemia: a potential target for ricin A chain immunotoxins. Blood 65:1416-1421, 1985.

84. Lambert, T.L, Kent, R.S. and Whorton, A.R. Bradykinin stimulation of inositol polyphosphate production in porcine aortic endothelial cells. J. Biol. Chem 261:15288-15293, 1986.

85. Larkin, J., Brown, M., Goldstein, J. and Anderson, R. Depletion of intracellular potassium arrests coated pit formation and receptor-mediated endocytosis in fibroblasts. Cell 33:273-285, 1983.

86. Leek, M., Griffiths, G. and Green, M. Intestinal pathology following intramuscular ricin poisoning. J. Pathol. 159:329-334, 1989.

87. Leith, A.G., Griffiths, G.D. and Green, M.A. Quantification of ricin toxin using a highly sensitive avidin/biotin enzyme-linked immunosorbent assay. J. Forensic. Sci. Soc. 28:227-236, 1988.

88. LeMaistre, C., Edwards, D., Krolick, K. and McGuire, W. An immunotoxin cytotoxic for breast cancer cells in vitro. Cancer Res. 47:730-734, 1987.

89. Lin, J., Liu, K., Chen, C. and Tung, T. Effect of crystalline ricin on the biosynthesis of protein, RNA and DNA in experimental tumor cells. Cancer Res. 31:921-924, 1971.

90. Lin, J., Tserg, K., Chen, C., Lin, L. and Tung, T. Abrin and ricin: New anti-tumour substances. Nature 227:292-293, 1970.

91. Locher, R., Neyses, L., Stimpel, M., Küffer, B. and Vetter, W. The cholesterol content of the human erythrocyte influences calcium influx through the channel. Biochem. Biophys. Res. Comm. 124:822-828, 1984.

92. Marks, A., Ettenson, D., Bjorn, M., Lei, M. and Baumal, R. Inhibition of human tumor growth by intraperitoneal immunotoxins in nude mice. Cancer Res. 50:288-292, 1990.

93. Martin, T.W. and Wysolmerski, R.B. Ca^{2+}-dependent and Ca^{2+}-independent pathways for release of arachidonic acid from phosphatidylinositol in endothelial cells. J. Biol. Chem. 26:13086, 1987.

94. Maxwell, R.A., Eckhardt, S.B. and Wastila, W.B. Concerning the distribution of endogenous norepinephrine in the adventitial and medial-intimal layers of the rabbit aorta and the capacity of these layers to bind tritiated norepinephrine. J. Pharmacol. Exp. Ther. 161:34, 1968.

95. Meisheri, L.D., Palmer, L.F. and van Breemen, C. The effects of amrinone on contractility, calcium intake and cAMP in smooth muscle. Eur. J. Pharmacol. 61:159-165, 1980.

96. Mosinger, M. Effets nécrosants ou clastiques exercés par la ricine sur divers organes et sur les sarcomes expérimentaux. Compt. Rend. Soc. Biol. 145:412-415, 1951.

97. Moya, M., Dautry-Varsat, A., Goud, B., Louvard, D. and Boquet, P. Inhibition of coated pit formation in Hep_2 cells blocks the cytotoxicicty of diphtheria toxin but not that of ricin toxin. J. Cell Biol. 101:548-559, 1985.

98. Nanno, S., Ishiguro, M., Funatsu, G. and Funatsu, M. The mode of binding of carbohydrate in ricin D. Agric. Biol. Chem. 39:1651-1654, 1975.

99. Naseem, S.M. and Pace, J.G. Effects of ricin on macromolecular synthesis and arachidonic acid metabolism in cultured macrophages. FASEB J. 5:Abstract 5229, 1991.

100. Naseem, S.M. and Pace, J.G. Effect of the steroidal and non-steroidal anti-inflammatory agents on ricin-mediated toxicity in cultured macrophages. The Pharmacologist 34:379, 1992.

101. Naseem, S.M., Wellner, R.B. and Pace, J.G. The role of calcium ions for the expression of ricin toxicity in cultured macrophages. J. Biochem. Toxicology. 7:133-138, 1992.

102. Nicolson, G. Ultrastructural analysis of toxin binding and entry into mammalian cells. Nature 251:628-630, 1974.

103. Nicolson, G. and Blaustein, J. The interaction of Ricinus agglutinin with normal and tumor cell surfaces. Biochim. Biophys. Acta 266:543-547, 1972.

104. Nicolson, G., Blaustein, J. and Etzler, M. Characterization of two plant lectins from Ricinus communis and their quantitative interaction with a murine lymphoma. Biochimistry 13:196-204, 1974.

105. Nicolson, G.L., Lacorbiere, M. and Hunter, T.R. Mechanism of cell entry and toxicity of an affinity-purified lectin from Ricinus Communis and its differential effects on normal and virus-transformed fibroblasts. Cancer Res. 35:144-155, 1975.

106. Oda, T. and Funatsu, G. Cross-linking of the two constituent polypeptide chains of ricin D with N,N'-*O*-Phenylenedimaleimide. Agric. Biol. Chem. 43:547-554, 1979.

107. Oladapo, J.M., and Goodall, [illegible] H., de Koning, R., Parman, J., Brown, D. and Thomas, H.C. In vitro and in vivo cytotoxic activity of native and ricin conjugated monoclonal antibodies to HBs antigen for Alexander primary liver cell carcinoma cells and tumours. [illegible]ut 25:619-623, 1984.

108. Olsnes, S., Fernandez-Puentes, C., Carrasco, L. and Vazquez, D. Ribosome Inactivation by the toxic lectins abrin and ricin. Eur. J. Biochem. 60:281-288, 1975.

109. Olsnes, S. and Pihl, A. Toxic Lectins and related proteins. in: The Molecular Actions of Toxins and Viruses (eds Cohen, P. and van Heyningen, S.) pp. 52-105 Elsevier Biomedical, New York, 1982.

110. Olsnes, S. and Pihl, A. in: The Specificity and Action of Animal, Bacterial and Plant Toxins (Cuatrecasas, P., ed), Vol. 1 pp. 131-173, Chapman and Hall, London. 1976.

111. Olsnes, S. and Refsnes, K. On the mechanism of toxin resistance in cell variants resistant to abrin and ricin. Eur. J. Biochem. 88:7-15, 1978.

112. Olsnes, S., Refsnes, K. and Pihl, A. Mechanism of action of the toxic lectins abrin and ricin. Nature 249:627-639, 1974.

113. Olsnes, S. and Sandvig, K. (1983) Entry of toxic proteins into cells. In: Receptors and Recognition, Series B, vol. 15 (Cuatrecasas, P., and Roth, T.F. eds.) pp. 189-236, Chapman and Hall Ltd., London.

114. Olsnes, S., Sandvig, K., Refsnes, K. and Pihl, A. Rates of different steps involved in the inhibition of protein synthesis by the toxic lectins abrin and ricin. J. Biol. Chem. 251:3985-3992, 1976.

115. Pace, J.G., Matson, C.F., Leggett, M., Bostian, K. and Rivera, E. Toxicokinetics of ricin in isolated perfused rat livers. The Toxicologist 12:Abstract 819, 1992.

116. Paulus, H., Machleder, H., Levine, S., Yu, D. and MacDonald, N. Lymphocyte involvement in rheumatoid arthritis. Studies during thoracic duct drainage. Arthritis Rheum. 20:1249-1262, 1977.

117. Pincus, S., Wehrly, K. and Chesebro, B. Treatment of HIV tissue culture infection with monoclonal antibody-ricin A chain conjugates. J. Immunology 142:3070-3075, 1989.

118. Pinto, A., Abraham, N.G. and Mullane, K.M. Cytochrome P-450-dependent monoxygenase activity and endothelial-dependent relaxations induced by arachidonic acid. J. Pharmacol. Exp. Ther. 236:445-451, 1986.

119. Press, O., Martin, P., Thorpe, P. and Vitetta, E. Ricin A chain containing immunotoxins directed against different epitopes on the CD2 molecule differ in their ability to kill normal and malignant T cells. J. Immunol. 141:4410-4417, 1988.

120. Raso, V., Ritz, J., Basala, M. and Schlossman, S. Monoclonal antibody-ricin A chain conjugate selectively cytotoxic for cells bearing the common acute lymphoblastic leukemia antigen. Cancer Res. 42:457-464, 1982.

121. Ready, M.P., Kim, Y. and Robertus, J.D. Site-directed mutagenesis of ricin A-chain and implications for the mechanism of action. Proteins: Structure, Function, and Genetics. 10:270-278, 1991.

122. Refsnes, K., Olsnes, S. and Pihl, A. On the toxic proteins abrin and ricin. J. Biol. Chem. 249:3557-3562, 1974.

123. Rico, I., Alonso, M.J., Salaices, M. and Marin, J. Pharmacological dissection of Ca^{2+} channels in the rat aorta by Ca^{2+} entry modulators. Pharmacol. 40:330-342, 1990.

124. Roth, J.A. Presence of membrane-bound catechol-*O*-methyltransferase in human brain. Biochem. Pharmacol. 29:3119-3122, 1980.

125. Roth, J., Ames, R., Fry, K., Lee, H. and Scannon, P. Mediation of reduction of spontaneous and experimental pulmonary metastases by ricin A-chain immunotoxin 45-2D9-RTA with potentiation by systemic monensin in mice. Cancer Res. 48:3496-3501, 1988.

126. Rotrosen, D. and Gallin, J.I. Histamine type I receptor occupancy increases endothelial cytosolic calcium, reduces F-actin, and promotes albumin diffusion across cultured endothelial monolayers. J. Cell Biol. 103:2379-2387, 1986.

127. Rutenber, E., Katzin, B.J., Ernst, S., Collins, E.J., Mlsna, D., Ready, M.P. and Robertus, J.D. Crystallographic refinement of ricin to 2.5 Å. Proteins: Structure, Function, and Genetics. 10:240-250, 1991.

128. Rutenber, E. and Robertus, J.D. Structure of ricin B-chain at 2.5 Å resolution. Proteins: Structure, Function and Genetics. 10:260-269, 1991.

129. Sandvig, K. and Olsnes, S. Effect of temperature on the uptake, excretion and degradation of abrin and ricin by HeLa cells. Exp. Cell Res. 121:15-25, 1979,

130. Sandvig, K. and Olsnes, S. Entry of the toxic proteins abrin, modeccin, ricin and diphtheria toxin into cells. I. Requirement for Calcium, and II. Effect of pH, metabolic inhibitors and ionophores and evidence for toxin penetration from endocytic vesicles. J. Biol. Chem. 257:7495-7513, 1982.

131. Sandvig, K., Olsnes, S. and Pihl, A. Binding, uptake and degradation of the toxic proteins abrin and ricin by toxin-resistant cell variants. Eur. J. Biochem. 82:13-23, 1978.

132. Sandvig, K., Tonnessen, T. and Olsnes, S. Ability of inhibitors of glycosylation and protein synthesis to sensitize cells to abrin, ricin, Shigella toxin and Pseudomonas toxin. Cancer Res. 46:6418-6422, 1986.

133. Seto, M., Umemoto, N., Saito, M., Masuho, T., Hara, T. and Takahashi, T. Monoclonal Anti-MM46 antibody: ricin A chain conjugate: In vitro and in vivo antitumor activity. Cancer Res. 42:5209-5215, 1982.

134. Shepherd, J.T. and Katusic, Z.S. Endothelium-derived vasoactive factors:I Endothelium-dependent relaxation. Hypertension 18[suppl III]:III-76-III-85, 1991.

135. Silverstein, S.C., Steinman, R.M. and Cohn, Z.A. Endocytosis. Annu. Rev. Biochem. 46: 669-722, 1977.

136. Simmons, B., Stahl, P. and Russell, J. Mannose receptor-mediated uptake of ricin toxin and ricin A chain by macrophages. J. Biol. Chem. 261:7912-7920, 1986.

137. Singer, H.A. and Peach, M.J. Calcium- and endothelial-mediated vascular smooth muscle relaxation in rabbit aorta. Hypertension 4 (supplII):19-25, 1982.

138. Singer, H.A. and Peach, M.J. Endothelium-dependent relaxation of rabbit aorta. 1. Relaxation stimulated by arachidonic acid. J. Pharmacol. Exp. Ther. 226:790-795, 1983.

139. Singer, H.A., Saye, J.A. and Peach, M.J. Effects of cytochrome P450 inhibitors on endothelium-dependent relaxation in rabbit aorta. Blood Vessels 21:223-230, 1984.

140. Skilleter, D.N. and Foxwell, B.M.J. Selective uptake of ricin A-chain by hepatic non-parenchymal cells in vitro. FEBS 196: 344-348, 1986.

141. Spitler, L., del Rio, M., Khentigan, A., Wedel, N., Brophy, N., Miller, L., Harkonen, W., Rosendorf, L., Lee, H., Mischak, R., Kawahata, R., Stoudemire, J., Fradkin, L., Bautista, E. and Scannon, P. Therapy of patients with malignant melanoma using a monoclonal antimelanoma antibody-ricin A chain immunotoxin. Cancer Res. 47:1717-1723, 1987.

142. Swauger, J.E., Pace, J.G., Glass, P.J., Rembish, S.J. and Trush, M.A. Effect of ricin on mitochrondrial function in alveolar macrophages. The Toxicologist, 12:Abstract 1089, 1992.

143. Thompson, W.L. and Pace, J.G. Studies on the use of brefeldin A and 3'-azido-3'-deoxythymidine to block the toxic effects of ricin *in vitro*. The Toxicologist 12:Abstract 524, 1992.

144. Thorpe, P.E., Detre, S.I., Foxwell, B.M., Brown, A.N.F., Skilleter, D.N., Wilson, G., Forrester, J.A. and Stirpe, F. Modification of the carbohydrate in ricin with metaperiodate-cyanoborohydride mixtures. Effects on toxicity and *in vivo* distribution. Eur. J. Biochem. 147:197-206, 1985.

145. Till, M., Ghetie, V., Gregory, T., Patzer, E., Poerter, J., Uhr, J., Capon, D. and Vitetta, E. HIV-infected cells are killed by rCD4-ricin A chain. Science 242:1161-1168, 1988.

146. Timar, J., McIntosh, D.P., Henry, R., Cumber, A.J., Parnell, G.D. and Davies, A.J.S. The effect of ricin B chain on the intracellular trafficking of an A chain immunotoxin. Br. J. Cancer. 64:655-662, 1991.

147. van Breemen, C. and Saida, K. Cellular mechanisms regulating $[Ca^{2+}]_i$ smooth muscle. Annu. Rev. Physiol. 51:315-329, 1989.

148. van Deurs, B., Sandvig, K., Petersen, O. and Olsnes, S. Endocytosis and intracellular sorting of ricin. in Trafficking of bacterial toxins. Saelinger, C. ed. CRC Press pp. 91-119, 1990.

149. van Deurs, B., Tønnenssen, T., Petersen, O., Sandvig, K. and Olsnes, S. Routing of internalized ricin and ricin conjugates to the Golgi complex. J. Cell Biol. 102:37-47, 1986.

150. Villafranca, J.E. and Robertus, J.D. Ricin B chain is a product of gene duplication. J. Biol. Chem. 256:554-556, 1981.

151. Wannemacher Jr., R.W., Hewetson, J.F., Lemley, P.V., Poli, M.A., Dinterman, R.E., Thompson, W.L. and Franz, D.R. (1992) Comparison of detection of ricin in castor bean extracts by bioassays, immunoassays, and chemistry procedures. In: Recent Advances in Toxinology Research, vol. 3 (Gopalakrishnakone, P. and Tan, C.K., editors) pp. 108-119, Venom and Toxin Research Group, Singapore.

152. Wannemacher Jr., R.W., Thompson, W.L. and Dinterman, R.E. Use of lactulose for prophylaxis/treatment of ricin (RCA_{60}) intoxication. FASEB J. 5:Abstract 2625, 1991b.

153. Wannemacher Jr., R.W., Thompson, W.L., Dinterman, R.E. and Grillo, F.G. In-vivo treatment of ricin intoxication. The Toxicologist 11:Abstract 498, 1991a.

154. Weiner, L., O'Dwyer, J., Kitson, J., Comis, R., Frankel, A., Bauer, R.L., Konrad, M. and Groves, E. Phase I evaluation of an anti-breast carcinoma monoclonal antibody 260F9-recombinant ricin A chain immunoconjugate. Cancer Res. 49:4062-4067, 1989.

155. Weiss, G.B. Cellular pharmacology of lanthanum. Annu. Rev. Pharmacol. 14:343-354, 1974.

156. Wiley, R.G., Blessing, W.W. and Reis, D.J. Suicide transport-destruction of neurons by retrograde transport of ricin, abrin and modeccin. Science 216:889-890, 1982.

157. Wrenn, S., Homcy, C. and Haber, E. Evidence for the β-adrenergic receptor regulation of membrane-bound catechol-*O*-methyltransferase activity in myocardium. J. Biol. Chem. 254:5708-5712, 1979.

158. Wurtman, R.J. and Axelrod, J. A sensitive and specific assay for the estimation of monoamine oxidase. Biochem. Pharmacol. 12:1439-1440, 1963.

159. Yoshida, T., Chen, C., Zhang, M. and Wu, H.C. Brefeldin A which disrupts the membrane recycling between ER and Golgi reduces cytotoxicity of ricin in vero ahd CHO cells. Cell Biology Meeting, November, Abstract 1118, 1989.

160. Yoshitake, S., Funatsu, G. and Funatsu, M. Isolation and sequences of peptic peptides, and the complete sequence of Ile chain of ricin D. Agric. Biol. Chem. 42:1267-1274, 1978.

161. Zenner, H. Selective killing of laryngeal carcinoma cells by a monoclonal immunotoxin. Ann. Otol. Rhinol. Laryngol. 95:115-120, 1986.

162. Zovickian, J., Johnson, V. and Youle, R. Potent and specific killing of human malignant brain tumor cells by an anti-transferrin receptor antibody-ricin immunotoxin. J. Neurosurg. 66:850-861, 1987.